PB80-226657

Proceedings of the Third USA-USSR Symposium on the Effects of Pollutants Upon Aquatic Ecosystems: Theoretical Aspects of Aquatic Toxicology

(U.S.) Environmental Research Lab.-Duluth
Grosse Ile, MI

Jul 80

U.S. Department of Commerce
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Environmental Protection
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EPA-600/9-80-034
July 1980

PB80-226657

Research and Development

EPA

Proceedings of the Third USA-USSR Symposium on the Effects of Pollutants Upon Aquatic Ecosystems

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/9-80-034	2.	3. RECIPIENT'S ACCESSION NO. PB 80 226657
4. TITLE AND SUBTITLE Proceedings of the Third USA-USSR Symposium on the Effects of Pollutants Upon Aquatic Ecosystems : Theoretical Aspects of Aquatic Toxicology		5. REPORT DATE July 1980 Issuing Date 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Environmental Protection Agency-USA Soviet Academy of Sciences-USSR		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Large Lakes Research Station Environmental Research Laboratory - Duluth Grosse Ile, Michigan 48138		10. PROGRAM ELEMENT NO. A30B1A 11. CONTRACT/GRANT NO. Joint US-USSR Project 02.02-13
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory - Duluth MN Office of Research and Development U.S. Environmental Protection Agency Duluth, Minnesota 55804		13. TYPE OF REPORT AND PERIOD COVERED Inhouse 14. SPONSORING AGENCY CODE EPA/600/03

15. SUPPLEMENTARY NOTES
Prepared in cooperation with the Institute for the Biology of Inland Waters, Soviet Academy of Sciences, Borok, Jaroslavl Oblast, USSR

16. ABSTRACT

The Joint US-USSR Agreement on Cooperation in the Field of Environmental Protection was established in May of 1972. These proceedings result from one of the projects, Project 02.02-13, Effects of Pollutants Upon Aquatic Ecosystems and Permissible Levels of Pollution.

As knowledge related to fate and transport of pollutants has grown, it has become increasingly apparent that local and even national approaches to solving pollution problems are insufficient. Not only are the problems themselves frequently inter national, but an understanding of alternate methodological approaches to the problem can avoid needless duplication of efforts. This expansion of interest from local and national represents a logical and natural maturation from the provincial to a global concern for the environment.

In general, mankind is faced with very similar environmental problems regardless of the national of political boundaries which we have erected. While the problems may vary slightly in type or degree, the fundamental and underlying factors are remarkably similar. It is not surprising, therefore, that the interests and concerns of environmental scientists the world over are also quite similar. In this larger sense, we are our brother's brother, and have the ability to understand our fellowman and his dilemma, if we but take the trouble to do so. It is this singular idea of concerned scientists exchanging views with colleagues that provides the basic strength for this project.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Freshwater Phosphorus Nitrogen Pesticides Fishes Stream Flow Toxicity	Bioassay Communities Phytoplankton Nutrients Waste Treatment Water Quality	Toxic Substances Macrobenthos Microbiota Water Quality Criteria Great Lakes Maximum Permissible Concentrations	57H 68D

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* unclassified	21. NO. OF PAGES
	20. SECURITY CLASS *(This page)* unclassified	22. PRICE

☆ U.S. GOVERNMENT PRINTING OFFICE:1980--657-165/0085

RESEARCH REPORTING SERIES

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EPA-600/9-80-034
July 1980

PROCEEDINGS OF THE THIRD USA-USSR SYMPOSIUM ON THE EFFECTS OF POLLUTANTS UPON AQUATIC ECOSYSTEMS

Theoretical Aspects of Aquatic Toxicology

July 2-6, 1979

Borok, Jaroslavl Oblast
USSR

Edited by

Wayland R. Swain
and
Virginia R. Shannon

ENVIRONMENTAL RESEARCH LABORATORY-DULUTH
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MINNESOTA 55804

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FOREWORD

These Proceedings result from the third symposium held by Project 02.02-13 under the aegis of the US-USSR Joint Agreement in the Field of Environmental Protection, established in May, 1972.

Both broad review and narrowly specific papers were presented by participants from both countries in an effort to continue the joint procedural, technological and methodological exchange and familiarization begun at the two preceeding symposia in 1975 and 1976. Learning does not occur *de novo* and subsequent understanding and application must be based on a foundation of fact. The atmosphere of mutual interest, candor and respect which surrounded this symposium enabled another series of steps in the learning process. Perhaps the philosphy underlying this symposium, and the project itself is best expressed by an old saying, which transliterated from the Russian approximates: Vyek zhee-vee, Vyek oo-chee, Live a lifetime, learn a lifetime.

Norbert Jaworski, Ph.D
Director
Environmental Research Laboratory-Duluth

PREFACE

This volume contains the papers presented at the Third US-USSR Symposium on the Effects of Pollutants on Aquatic Ecosystems entitled, "Theoretical Aspects of Aquatic Toxicology". All of the papers were presented in English or Russian with simultaneous translations into the corresponding language at Borok, Jaroslval Oblast, USSR during July 2-6, 1979, at the Institute for the Biology of Inland Waters of the USSR Academy of Sciences.

Professor N.V. Butorin, Director of the Institute and Project Leader for the Soviet side, served as official host for the American delegation and has assumed the responsibility for the publication of these proceedings in the Russian language. This joint bilingual publication represents a reaffirmation of the continuing commitment pledged by both countries to cooperative environmental activities.

INTRODUCTION

The Joint US-USSR Agreement on Cooperation in the Field of Environmental Protection was established in May of 1972. These proceedings result from one of the projects, Project 02.02-13, Effects of Pollutants Upon Aquatic Ecosystems and Permissible Levels of Pollution.

As knowledge related to fate and transport of pollutants has grown, it has become increasingly apparent that local and even national approaches to solving pollution problems are insufficient. Not only are the problems themselves frequently international, but an understanding of alternate methodological approaches to the problem can avoid needless duplication of efforts. This expansion of interest from local and national represents a logical and natural maturation from the provincial to a global concern for the environment.

In general, mankind is faced with very similar environmental problems regardless of the national of political boundaries which we have erected. While the problems may vary slightly in type or degree, the fundamental and underlying factors are remarkably similar. It is not surprising, therefore, that the interests and concerns of environmental scientists the world over are also quite similar. In this larger sense, we are our brother's brother, and have the ability to understand our fellowman and his dilemma, if we but take the trouble to do so. It is this singular idea of concerned scientists exchanging views with colleagues that provides the basic strength for this project. While our methods may vary, our goals are identical, and therein lies the value of such a cooperative effort.

Wayland R. Swain, Ph.D., and
Richard A. Schoettger, Ph.D.
Co-Project Leaders, U.S. Side

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ACKNOWLEDGMENTS

In any project of the scope and complexity of this effort, the Project Officers become increasingly indebted to a large number of individuals who contribute their time and effort with no thought of personal gain. Unfortunately, the list of persons who materially aided the effort is too extensive to allow a complete discussion. However, while those persons who made outstanding contributions to the success of this project are acknowledged below, the editors also wish to thank all those others, both Soviet and American, whose efforts and assistance smoothed the way to a satisfactory completion of this phase of the project.

Sincere thanks are extended for the considerable efforts, patience and support of Gary Waxmonsky and Jean MaGuire of the U.S. Executive Secretariat of the US-USSR program. Their assistance and prompt attention to the details of translations of texts, movement of equipment, international cable traffic and travel clearances enabled the meetings of the U.S. personnel with Soviet counterparts, and facilitated the preparation of this report.

The many contributions of Ms. Nina Ivanikiw to the preparation of both the visit to the Soviet Union and to the coordination and preparation of materials for this publication are remembered with deep appreciation.

The substantial contributions and tireless efforts of Ms. Debra Caudill to the preparation of these proceedings are gratefully acknowledged.

To the many Soviet colleagues, friends, and acquaintances who labored so diligently to make the Borok symposium such a success, and the visit of the eleven participants to Siberia and Lake Baikal so memorable, we offer profound thanks, Большой Спасибо!

SECTION 1

A RESEARCH STRATEGY FOR ANTICIPATING CONTAMINANT THREATS TO AQUATIC RESOURCES

Richard A. Schoettger and J. Larry Ludke[1]

The Environmental Contaminant Evaluation Program of the United States Fish and Wildlife Service (USFWS) is emphasizing a predictive approach to identify potential contaminant problems and preventing or ameliorating adverse effects of contaminants on ecological systems. The primary objective is to protect fishery and wildlife resources from the impacts of contaminants before the effects become irreversible, or reversible only with great difficulty and at high cost. Predictive research has long been a priority objective of USFWS work with environmental contaminants. For example, DDE was shown to cause reduction in avain populations; exposure to this chemical resulted in thinned eggshells, which decreased the production of offspring. Although these effects were repeatedly demonstrated in laboratory experiments, regulatory action to remedy the problem was not taken for several years.

Contaminant problems of the 1970's, however, overwhelmed the research capability to address them, and predictive research fell behind in the midst of pressures to solve current problems. A new thrust was initiated in 1977 to increase USFWS capability to anticipate contaminant threats to the nation's fishery and wildlife resources. The intent of this renewed emphasis is to increase the base of knowledge and thus assist natural resource managers in anticipating and addressing future or suspected contaminant problems before they reach catastrophic proportions.

Because manpower and scientific resources are limited, we in the environmental research community must emphasize the necessity of placing priorities on our fishery and wildlife resources. We must judge on the relative importance of different species and habitats on the basis of uniform and meaningful guidelines, and focus our efforts on protecting the most important ones first. Such an effort necessarily involves a multidisciplined approach with a goal of anticipating contaminant threats of the future.

The Columbia National Fisheries Research Laboratory (CNFRL) has employed a strategy that accentuates the anticipation of new or previously unrecognized pollution problems, while continuing to address old problems that remain a concern (Figure 1). The approach draws upon a number of different sources to assist in the identification of present and potential contaminant effects. It is actually little more than application of the logic of the scientific method. Information and data that relate to topics of concern are reviewed by scientists and resource managers to develop an overview of a problem and to determine data needs. A research design is then formulated to provide information on the real or potential effects a contaminant may have on aquatic organisms or ecosystems. From the results of such research, we may often be able to make remedial recommendations. Corrective or preventive alternatives that include one or more of the following may then be recommended:

---

[1] Columbia National Fisheries Research Laboratory, U.S. Department of the Interior, Fish and Wildlife Service, Route #1, Columbia, Missouri 65201.

a) legislative action to regulate or prohibit the manufacture, use, or disposal of a chemical,

b) modification of management techniques or practices to protect fish or other aquatic resources from the contaminant,

c) changes in the development, use or application of certain chemicals,

d) suggested substitute chemicals which prove less harmful,

e) selection of a less harmful activity or process over one that is proven deleterious.

Our strategy insures that resource managers are involved in the process of problem identification and formulation of research design, so that the objectives and results are applicable to the actual environmental problems that confront the aquatic resources. It also assures consideration of the most vulnerable resources that may be impacted by a contaminant.

The key to applying this strategy successfully at the national level is to simultaneously identify the most critical resources of concern and the activities and contaminants most likely to adversely affect those resources. Limited funds and manpower dictate the necessity of identifying the most critical or vulnerable biota and habitat that may be affected by any contaminant or polluting activity of man. This identification requires that we develop a comprehensive inventory of resources and habitat under our protection. We must distinguish between localized problems and those that are widespread. Problems of short duration (e.g., one-time occurrences) or those which are in the process of remediation must be recognized, but research emphasis must be oriented toward long-term contaminant problems that have potentially devasting impacts in the foreseeable future.

It has been estimated that the number of potential chemical contaminants that may pollute U.S. lakes and streams could exceed 87,000. There are 129 priority toxic substances listed by the U.S. Environmental Protection Agency (EPA) for immediate assessment of production, distribution, disposal, toxicity, fate within the environment, and ecological impacts. Hundreds more of these chemicals are awaiting ecological hazard evaluation. Though some of

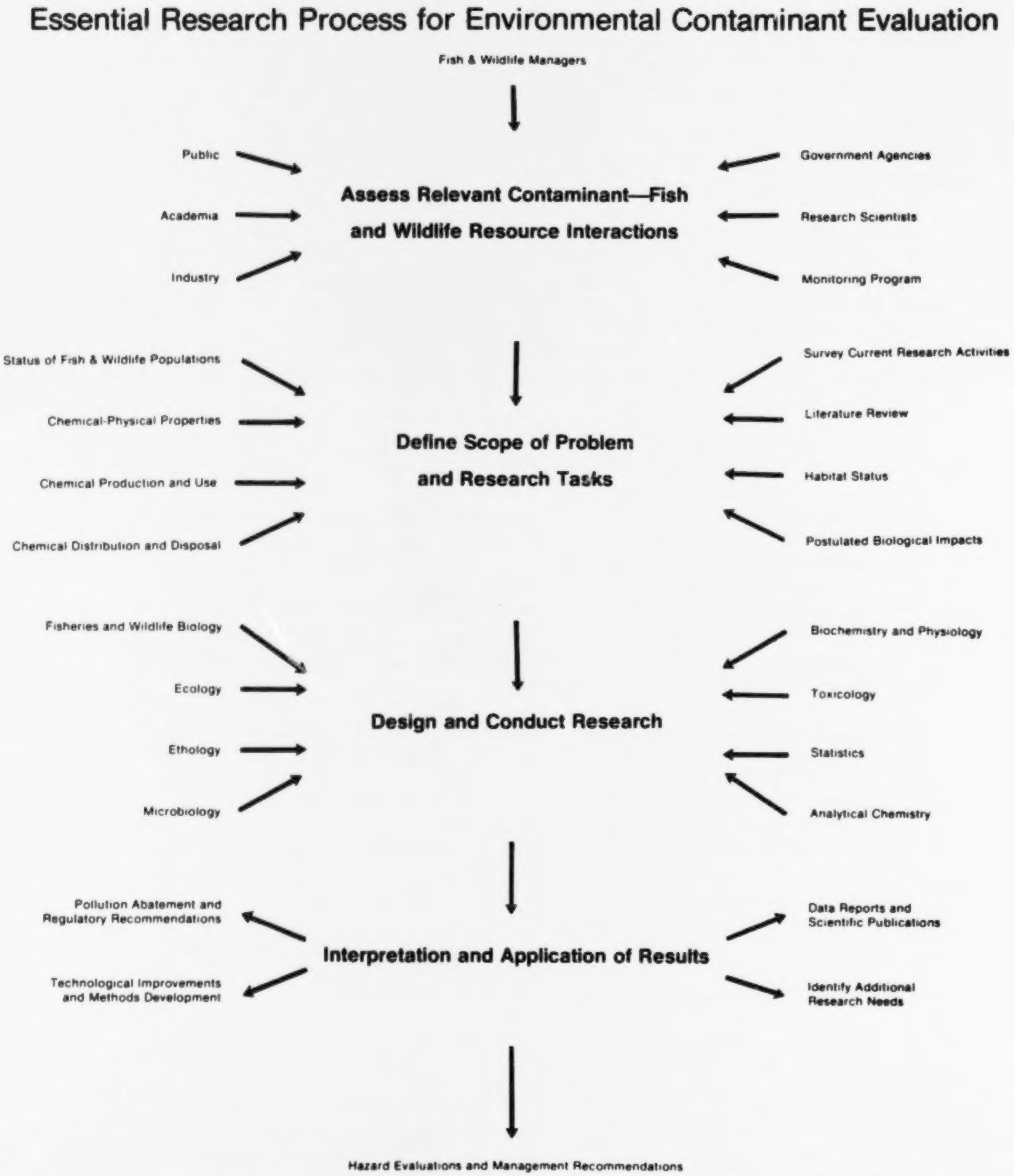
Essential Research Process for Environmental Contaminant Evaluation
Fish & Wildlife Managers
Public
Academia
Industry
Assess Relevant Contaminant—Fish and Wildlife Resource Interactions
Government Agencies
Research Scientists
Monitoring Program
Status of Fish & Wildlife Populations
Chemical-Physical Properties
Chemical Production and Use
Chemical Distribution and Disposal
Define Scope of Problem and Research Tasks
Survey Current Research Activities
Literature Review
Habitat Status
Postulated Biological Impacts
Fisheries and Wildlife Biology
Ecology
Ethology
Microbiology
Design and Conduct Research
Biochemistry and Physiology
Toxicology
Statistics
Analytical Chemistry
Pollution Abatement and Regulatory Recommendations
Technological Improvements and Methods Development
Interpretation and Application of Results
Data Reports and Scientific Publications
Identify Additional Research Needs
Hazard Evaluations and Management Recommendations

Figure 1. Major steps and some sources of input for a research approach to assessing contaminant threats to fish and wildlife resources.

the needed information is available for hazard assessment, as stewards of the nation's biological resources, the USFWS must increase its efforts in determining which of the many pollutants are reaching or may reach the resources we are charged with protecting. To make this determination, the Service is developing its priorities, emphasizing the resources that can least afford to be lost. If a contaminant or polluting activity is not likely to affect a priority resource, we need not waste valuable time and effort in studying it.

We now have all of the components for a framework to address environmental contaminant impacts on living resources. Implementation of the approach requires that the components be placed together in a logical sequence to achieve proper perspective, set priorities, and then act. Conceptually, we progress through a logical continuum of four steps: a) problem identification, b) definition of scope of problem, c) research to provide data or fill information gaps, and d) interpretation and application of results.

Information elucidating potential contaminant problems that threaten the well-being of fish and wildlife resources come from a variety of sources:

1. Resource managers - Federal and state management personnel identify contaminant problems, often from obserservation of mortality of fish or wildlife in the environment. Declines in populations may be observed and reported. Through residue surveys or in concert with USFWS Research monitoring activities, "hot spots" are identified. Follow-up research studies are initiated to elucidate the full scope and effects of observed problems.

2. Other government agencies - The most obvious source of input suggesting contaminants of concern comes from the EPA. Under the Toxic Substances Control Act, EPA is charged to "regulate commerce and protect human health and the environment by requiring testing and necessary use restrictions on certain chemical substances...". The total of 129 priority compounds now on the EPA toxic substance list for environmental hazard evaluation includes the following major chemical families: chlorinated benzenes, chlorinated naphthalene, haloethers and halomethanes, nitrophenols, phthalate esters, nitrosamines, polynuclear aromatic hydrocarbons, organochlorine pesticides, polychlorinated biphenyls, and selected metals.

3. Research scientists - Scientists who are expert in research on environmental contaminant effects have particularly valuable insight regarding contaminants that require further study. Observations and results obtained through carefully planned research often provide the researcher only with parts of the answer being sought. New questions arise which may be answered by further research to provide additional insight.

4. Industry - Industry can be, and often is, a contributing participant in identifying potential contaminants that must be assessed before they are marketed. CNFRL has worked closely with one chemical company in initial toxicity assessment of compounds that are being considered as PCB replacements. Results have been encouraging, and we believe this working relationship between government and industry to be highly desirable.

There are other sources from which we get leads or indications as to the contaminants of highest potential concern (e.g., academia, monitoring programs, conservation groups, etc.).

The important point is that there is no paucity of contaminants and contaminant problems. The possibilities far exceed our potential in manpower, funds, and time to address them in detail. So it is incumbent upon us to identify and locate the populations and habitats that are most important to us, whether they be highly vulnerable and pristine, threatened or endangered, or of sport, commercial or aesthetic value. Only by ordering our resources into categories of priority can we assess the relevancy and scope of contaminant-resource interactions, and thereby make more meaningful management and research decisions. It does not matter whether the potential contaminant is an organophosphate, a dioxin, toxaphene, or crude oil. What does matter is whether that substance will adversely affect, directly or indirectly, a valued resource.

Traditionally, we have oriented our efforts toward studying the chemical and its effects under highly controlled conditions. Emphasis has been on anticipating contaminants which may have highly detrimental effects because of their toxicity, distribution, or disposal. We are now putting greater emphasis on assessing the resource-contaminant interaction. We want to better consider the potential availability of the toxic contaminant to the fish and wildlife resources that have been identified as being of high priority.

PCBs are known pollutants of the Upper Mississippi River, and in some areas their residues are alarmingly high. In 1971, commercial fishermen harvested 31.5 million pounds of fish from this productive stream. The extent, distribution and ecological significance of PCB residues in prime fish and diving duck habitats of the Upper Mississippi River have not yet been determined. Our field laboratory at LaCrosse, Wisconsin, is undertaking studies to describe the movement and fate of PCBs in productive fishery and wildlife habitat downstream from a major municipal source. Toxicity and bioconcentration of PCBs in aquatic biota is being studied to assess the relative hazard of these contaminants in the environment.

Through contact with fish and wildlife management personnel, our field research scientists are focusing on several broad areas of concern with respect to contaminant problems. Some of the topics relate to energy, including petroleum pollution, but numerous non-energy related contaminant poblems also require attention.

Ongoing work at CNFRL includes considerable effort in continued acute and chronic toxicity testing (Figure 2), monitoring and surveillance of contaminants in the environment, and continued methods development in analytical chemistry to better enable us to identify and quantitate a wide spectrum of contaminants in the environment. We are placing additional emphasis on ecosystem approaches, behavior studies, highly sophisticated analytical approaches to identify unknown contaminants in the environment, and assessment of biological or biochemical indicators of contaminant stress.

Contamination of the aquatic environment by agricultural and industrial chemicals, oil spills, mine effluents, and other forms of pollution has been recognized for many years. Evaluating the impact of the many contaminants on aquatic organisms has been limited mainly to short-term laboratory studies. Only recently have long-term laboratory studies been used to evaluate growth, reproduction, mortality and residue dynamics in relation to the environment. Although these studies strongly indicate safe toxicant concentrations, their disadvantages include the length of time required to complete partial and chronic toxicity studies, cost, and the limited number of aquatic species that can be cultured in laboratory or artificial environments. Much of the laboratory research lacks field verification, and the true impact of chemical contaminants on aquatic organisms in the natural environment is poorly understood. New techniques are needed that can be used as biological indicators or predictors in both laboratory and field investigations for estimating the health or status of a particular resource.

Development and validation of analytical capabilities must accompany laboratory studies dealing with the toxicological effects of contaminants. New analytical procedures have been implemented for di-2-ethylhexyl phthalate, pentachlorophenol, mirex, and Kepone in water and fish, and for mixed Arochlors (PCBs) in sediments from the Upper Mississippi River. The use of adsorbents has greatly facilitated the analysis of certain trace organics and led to the development of a new multichromatographic material that may permit one-step purification of many aromatic compounds, including dioxins and dibenzofurans.

Routine methods currently used in monitoring and surveillance programs enable us to measure fewer than 50 kinds of residues in fish. Thus, it is essential to develop a comprehensive strategy to detect and measure contaminants in fish and other sample material. Recent advances in chemical detection, sample extraction, and clean-up procedures make it possible to identify and quantitate a greater number of the components that make up the complex contaminants in aquatic systems.

Techniques are under development to fractionate complex mixtures of contaminants present in samples from aquatic environments into classes of chemicals to simplify the detection and to provide more comprehensive residue data (Figure 3). By using advanced scientific instruments, such as the mass spectrometers and the inductively coupled plasma emission spectrophotometer, we are gaining the ability to perform comprehensive analyses with much greater precision and accuracy. Separations of contaminants into classes, combined with new instrumentation, have helped identify several

Figure 2. Laboratory evaluation of chronic effects of contaminants on fish in a flow through diluter system.

Comprehensive Scheme for Cleanup, Fractionation, and Analysis of Environmental Contaminants

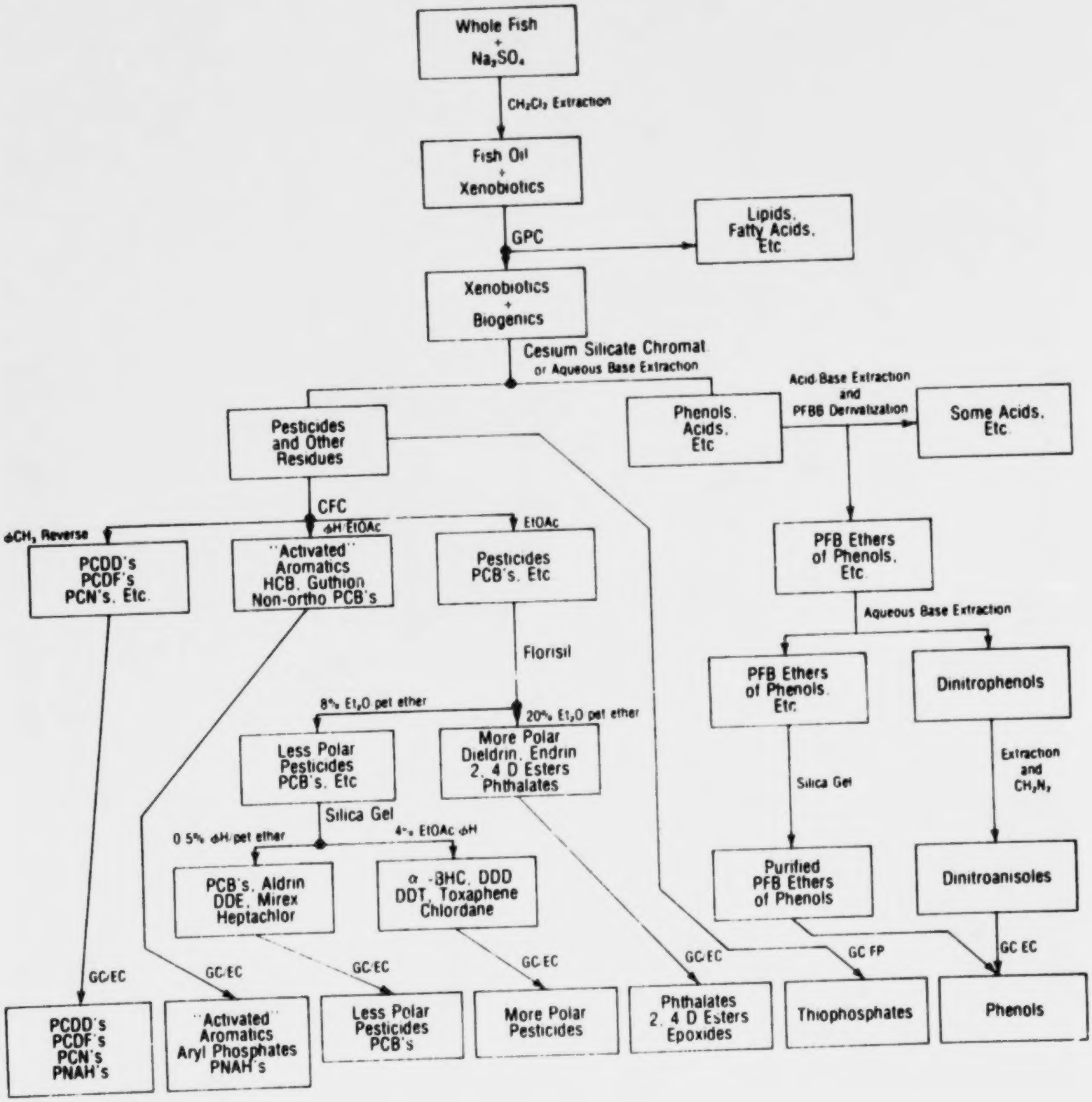
Whole Fish + Na_2SO_4
CH_2Cl_2 Extraction
Fish Oil + Xenobiotics
GPC
Lipids, Fatty Acids, Etc
Xenobiotics + Biogenics
Cesium Silicate Chromat or Aqueous Base Extraction
Pesticides and Other Residues
Phenols, Acids, Etc
Acid/Base Extraction and PFBB Derivatization
Some Acids, Etc
CFC
ϕCH_3 Reverse
ϕH/EtOAc
EtOAc
PCDD's PCDF's PCN's, Etc.
"Activated" Aromatics HCB, Guthion Non-ortho PCB's
Pesticides PCB's, Etc
Florisil
8% Et_2O pet ether
20% Et_2O pet ether
Less Polar Pesticides PCB's, Etc
More Polar Dieldrin, Endrin 2, 4 D Esters Phthalates
Silica Gel
0.5% ϕH/pet ether
4% EtOAc ϕH
PCB's, Aldrin DDE, Mirex Heptachlor
α-BHC, DDD DDT, Toxaphene Chlordane
PFB Ethers of Phenols, Etc
Aqueous Base Extraction
PFB Ethers of Phenols, Etc
Dinitrophenols
Silica Gel
Extraction and CH_2N_2
Purified PFB Ethers of Phenols
Dinitroanisoles
GC/EC
GC/EC
GC/EC
GC/EC
GC/EC
GC/FP
GC/EC
PCDD's PCDF's PCN's PNAH's
"Activated" Aromatics Aryl Phosphates PNAH's
Less Polar Pesticides PCB's
More Polar Pesticides
Phthalates 2, 4 D Esters Epoxides
Thiophosphates
Phenols

Figure 3. A comprehensive analytical schematic for the separation and analysis of organic contaminants.

previously unknown contaminants. Once contaminants are identified, needed toxicity data can be gathered to assess their impact on resources.

We have recently added to our professional staff eight fishery biologists who are located in major watershed regions of the United States (Figure 4). These scientists are working with toxicologists at our laboratory and with federal and state fishery and wildlife resource managers to identify present contaminant problems and potential contaminant threats of the future. The field biologists have been working to place contaminant problems of the present and future into perspective for planning and accomplishing research needed to assess contaminant hazards to natural resources. Contaminant problems associated with new or intensified activities of the future are undoubtedly numerous.

Many possible threats exist to wildlife and fish from activities in energy development. Although many of the activities are not new, their projected intensity is far greater than once expected. We have much to learn about the impacts of these activities on the environment.

The development, transport, or use of gas, coal, oil and oil shale could have substantial impact on the environment, particularly in the western United States where ecosystems have a low resiliency to ecological perturbation. Any material present in the crude energy source or used in the conversion to usable energy is a potential pollutant. Projected coal gasification and liquefaction plants and oil shale retorting facilities of the 1980's will result in a new area of contaminants associated with energy production. At this point, we can speculate on the identity of some of these potential contaminants, on the basis of existing technology in the analysis of crude oil and the by-products of conventional coal combustion. Toxic phenols, cresols, and water-soluble aromatics are high on the list of potential troublemakers. Certain aromatics of higher molecular weight (e.g., benzo-pyrene, benzanthracene, and naphthalene) are known carcinogens. A new generation of organometallics will be associated with coal conversion.

During exploratory drilling and production at petroleum wells, large amounts of water must be disposed of. In addition to metallic salts, the water contains numerous organic compounds derived from underlying petroleum pools. Much of this waste water is being dumped into freshwater streams and estuaries.

The "shopping list" of contaminant problems associated with energy is extensive. The Columbia National Fisheries Research Laboratory has initiated research in energy-related subjects that have been identified as being of high priority.

In many parts of the world, precipitation is becoming polluted with strong acids, trace elements, and complex organics. The major sources of these contaminants appear to be combustion of fossil fuels. Trace elements and organic compounds have not been routinely sampled in the past. However, some 450 organic contaminants including PCBs, DDT, polycylic aromatic hydrocarbons, and others, have been detected in precipitation.

COLUMBIA NATIONAL FISHERY RESEARCH LABORATORY
WITH
FIELD FACILITIES

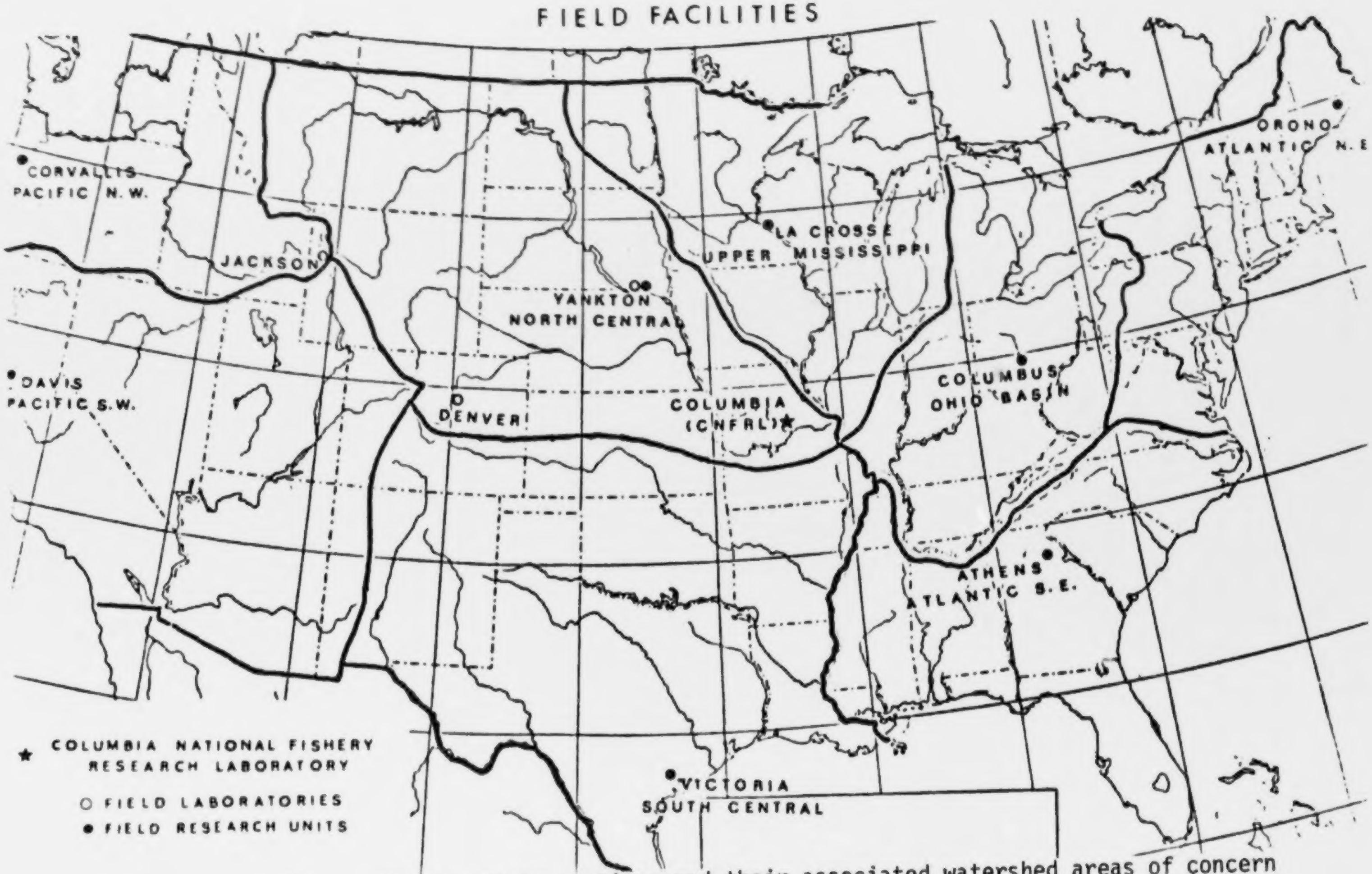
CORVALLIS
PACIFIC N.W.
JACKSON
DAVIS
PACIFIC S.W.
DENVER
YANKTON
NORTH CENTRAL
LA CROSSE
UPPER MISSISSIPPI
COLUMBIA
(CNFRL)
COLUMBUS
OHIO BASIN
ORONO
ATLANTIC N.E.
ATHENS
ATLANTIC S.E.
VICTORIA
SOUTH CENTRAL
★ COLUMBIA NATIONAL FISHERY RESEARCH LABORATORY
○ FIELD LABORATORIES
● FIELD RESEARCH UNITS

Figure 4. Locations of CNFRL field stations and their associated watershed areas of concern (outlined by heavy lines).

Prevailing weather patterns are such that the northeastern U.S. is subject to extensive fallout of acid and metals in precipitation (Figure 5). Most of the acid apparently originates over the industrial Midwest. Trace elements are higher in precipitation in the Northeast and Midwest or West than elsewhere. Halogens, mercury, selenium, arsenic and antimony are volatilized during coal combustion and many of the organic compounds identified in precipitation are the same as those found in some fuels.

Direct addition of acid from precipitation has caused a marked decline in pH of lakes and streams in Scandanavia; Ontario, Canada; and the Adirondak Mountains of New York. In many lakes in the Adirondaks, where the water is poorly buffered, pH ranged from pH 6.0-7.5 in the 1930's, but is commonly less than 5.0 today. Lowered pH renders most heavy metals more soluble and potentially more toxic to aquatic biota. Concentrations of mercury, copper, cadmium, nickel, lead and zinc have been shown to be higher in lakes affected by polluted precipitation than in others. Lowered pH also promotes increased leaching of naturally occurring metals (e.g., aluminum) from soils.

Surveys of lakes indicate that fish populations are virtually absent in waters with a pH below 5.5. Recent evidence indicates that lowland lakes are decreasing in buffering capacity and small headwater streams may be affected, particularly during spring melts.

There is a critical need for more information about the extent and distribution of polluted precipitation and its effects on lakes and streams. There is currently a lack of information on the chemistry and fish populations of vulnerable lakes in New England. The CNFRL field research unit at Orono, Maine, is beginning a study to correlate the pH, and metal content of lakes believed to be impacted in the northeastern United States. Diatom analysis will be used to document the history of pH changes. Fish populations will be surveyed for species composition and age distribution. Fish will be subjected to analysis for aluminum, arsenic, cadmium, copper, lead, silver, zinc, antimony and mercury.

Our objectives are (a) to determine recent history of pH and metal content of selected New England lakes, (b) to determine the chronology of fish population changes, (c) to correlate the heavy metal content with acid polluted lakes, and (d) to determine water quality changes in headwater streams in northern New England at spring thaw.

The United States has vast coal reserves in the West. Most of the reserves used over the next 20 years will be taken by surface mining. Some of it will be transported to points throughout the country, where it will be converted to usable energy. However, much of it will be converted to electric power at coal-fired power plants near mining sites, and the electricity transported to the user (Figure 6). The energy output of coal-fired facilities in Montana, Wyoming and the Dakotas will increase almost threefold between 1977 and 1985. The distribution and effects of airborne contaminants on aquatic and terrestrial systems are largely unkown. Questions that need to be answered include such items as the manner and degree that trace inorganics and organics cycle in the environment; the kinds of trans-

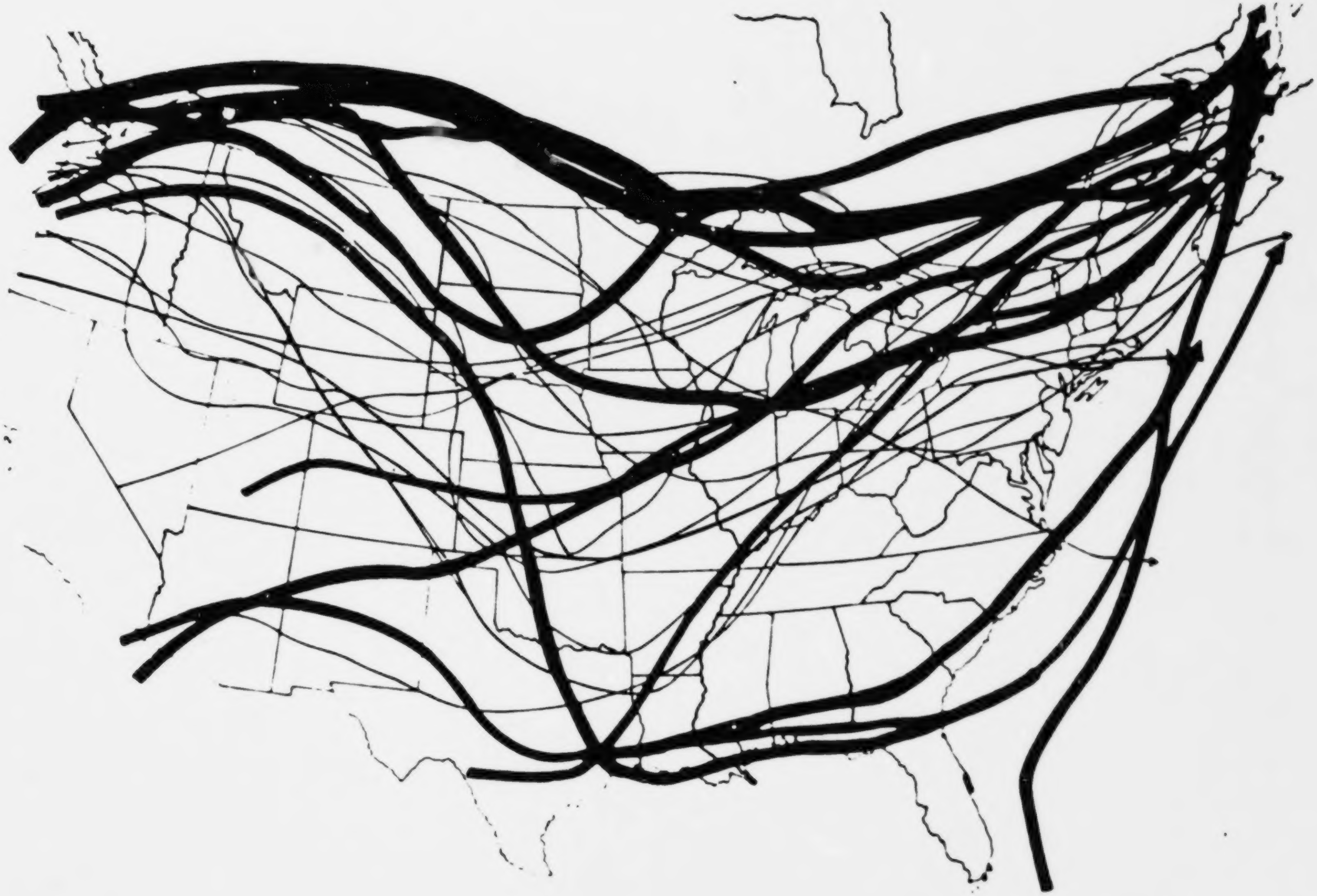

Figure 5. Storm tracks which indicate where acids and metals are deposited by precipitation in poorly buffered lakes and streams of New England.

Figure 6. One of many coal-fired power plants under construction in the Northern Great Plains of the U.S.

formations elements undergo as they cycle from air into water and biota; and the availability and toxicity of the trace contaminants that do penetrate to the aquatic system.

The Field Research Station at Victoria, Texas, in cooperation with the Texas Parks and Wildlife Department, conducted acute toxicity tests of oil-produced brine water to several estuarine fishes. Brine water from oil wells located near coastal areas of Texas are generally discharged into estuaries. An increase in the concentration of brine was followed by an increase in death rates of test organisms. Organisms tested in synthetic sea salt at the same salinity as the brine concentration showed a much lower death rate. Evidently some toxic component of the oil is dissolved in the brine, or the brine is interacting with the oil to increase toxicity. Further research at Victoria will include testing the effects of oil-produced brine water to standing crops and diversity of stream organisms. Increased salinity in Oklahoma streams has been traced to improperly capped wells and faulty injection casings; field research is planned to assess the impact of the increased salinity.

The pressures of oil shortages and deregulation of oil prices will result in additional exploration and development of new oil reserves and increased production from existing ones. Public lands in the mountainous areas of the western U.S. have been targeted as sites for new production. In active oil fields, large volumes of water are produced with crude oil. Water is separated from the oil and then reused or discharged. The limit of "oil and grease" discharge allowable is 10 parts per million (ppm) (Figure 7). No information has been generated to allow a proper hazard evaluation of these tolerated levels.

The CNFRL Field Research Laboratory at Jackson, Wyoming, conducted 90-day exposures of cutthroat trout to water soluble components of Wyoming Green, one of the major crude oil types produced in that area. At test concentrations of 0.6 ppm (less than one-tenth the allowable effluent concentration) trout mortality was 48% and growth was reduced by 88%. Growth of trout treated with as little as 0.1 ppm was reduced by 20%, and extensive fin erosion occurred. Avoidance studies have demonstrated that cutthroat trout are attracted to oil concentrations in water that also result in reduced growth and survival.

Numerous other contaminant threats to important aquatic resources have been identified. Some problems are of concern because they are ubiquitous, whereas others may affect specific isolated resources that are highly valued and especially vulnerable to contaminant stresses.

Millions of acres of riparian habitat have been degraded or destroyed by water resource projects over the past 50 years (Figure 8). Much of the destruction results from restriction of annual overflows of natural wetland areas. Overflow restriction has encouraged extensive land clearing and disrupted the normal hydrologic regime and water fluctuations in headwaters and backwater lakes and swamps. Flood control practices have destroyed hardwood forests and degraded once productive aquatic habitats, allowing these areas to be cleared and used for agriculture. Sediments and associated contami-

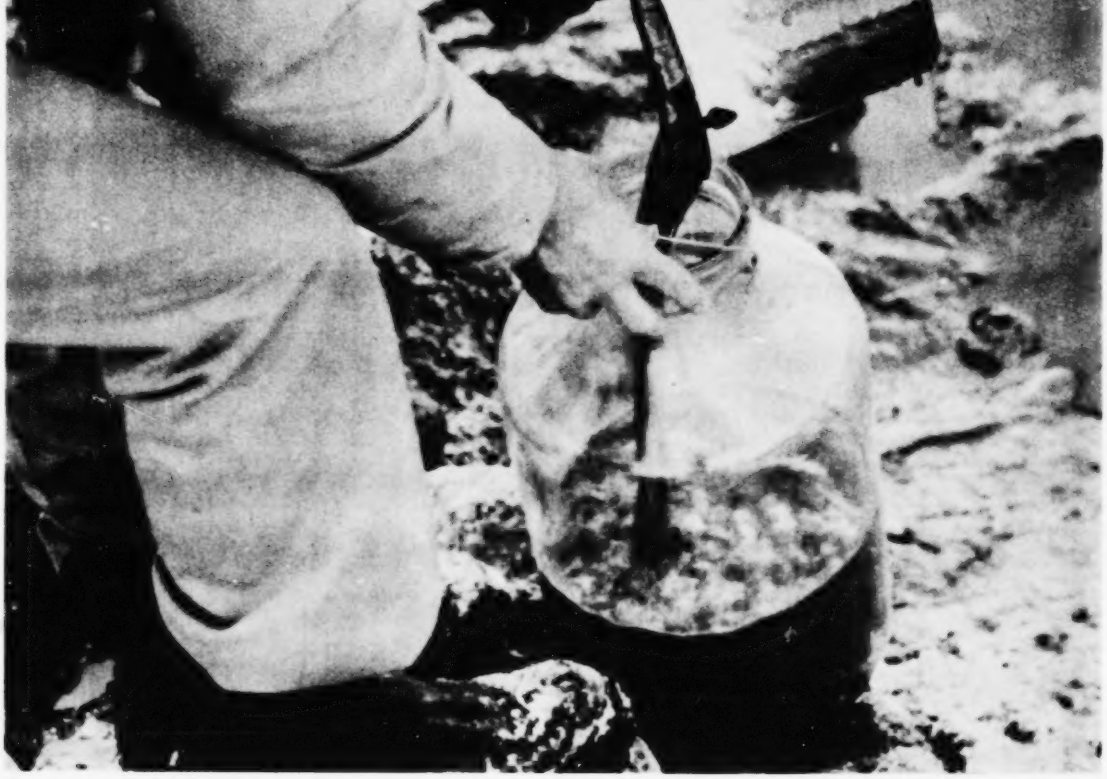

Figure 7. Researchers collecting water containing waste oil from a drilling operation in Wyoming. This discharge has been shown to be toxic to cutthroat trout.

Figure 8. Extensive clearing of irreplaceable bottomland hardwood forests. After cleared areas are completely drained they are usually converted to agriculture which causes increased sedimentation and contamination of fisheries by agricultural chemicals.

nants further degrade lakes that become surrounded by agricultural land. Even systems receiving annual overflow are being degraded by agricultural pollutants stemming from land-use activities. Though the literature is replete with qualitative information expounding the value of wetland systems, there is a paucity of quantitative information describing the effects of reduced overflow and contaminant effects on these ecosystems. Such information is needed to verify and document the effects of flood control activities (damming, channelization, diking, levee construction, etc.) and agricultural chemical impacts resulting from land use changes.

Other environmental contaminant problems of potentially serious consequence include the following:

a) Impact of contaminants in irrigation return waters on the anadromous fishes of the San Joaquin and Sacramento Rivers in the Central Valley of California;

b) Widespread toxaphene contamination of freshwater fisheries from increased use and atmospheric transport of the chemical;

c) Extensive use of herbicides in agriculture and silviculture;

d) Accumulation and chronic toxic effects of relatively unstudied industrial contaminants;

e) Continuing contamination of the environment by PCBs, dibenzofurans, and dioxins.

The proper evaluation of contaminant impacts of living resources involves a multidisciplined approach with input from scientisits, resource managers, industry, and academia. Matching the locations of more serious contaminant problems with areas of high resource value can serve as a guideline for directing limited research resources to properly assess contaminant threats or hazards to the environment. Researchers and resource managers can then work together to recommend approaches to identify and avoid or mitigate serious contaminant impacts on the environment.

SECTION 2

PRINCIPLES OF ESTIMATION OF NORMAL AND PATHOLOGIC STATES OF RESERVOIRS WITH CHEMICAL POLLUTION

N.S. Stroganov[1]

A need has been demonstrated for giving hydrobiologic principles priority over other principles in the evaluation of the status of a reservoir. The starting point for development of principles for evaluation is the need to preserve pure water in the reservoir, in which valuable commercial organisms can exist for long periods of time, and for fresh reservoirs, suitable also for supplying potable water. A reservoir which has water of this quality can be considered normal, one which does not have these qualities must be considered pathologic. Unless man's use of the water is brought into the picture, there is no foundation for speaking of the degree of normality of reservoirs.

The degree of pathology may differ. Selection of the species of aquatic organisms to be protected by man will be determined primarily by the functional significance of the species in the cycle of matter in the aquatic ecosystem, assuring good water quality and high productivity of valuable commercial species.

For water toxicology, theoretically, scientific determination of the limits of permissible changes in hydrobiologic processes in an organism is of great importance.

The increase in man's effect on nature (Bernadskiy 1967), including surface reservoirs and streams, has set for mankind a number of new problems which must be solved as quickly as possible. Man began influencing nature long ago. Ecologic crises have occurred in the past (Budyko 1977), but they have become particularly striking in certain regions since the 1940s. The situation has deteriorated to the point that the outlook of many toward the relationship of man and nature is quite pessimistic. We hear predictions of ecologic catastrophes (Douglas 1975), and various plans are set forth to avoid such catastrophes (Medouz, et al. 1972), and thus, the ecologic crises are denied for the present time (Budyko 1977). The disruption of equilibrium between man and nature is real. While it should not be drawn in emotional terms, there are rational means for solution of the problem. Probably the greatest of all problems with which society has ever wrestled (Oldak

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1979), must be addressed. Degradation of the environment and the advent of the ecologic catastrophe must be prevented. The biosphere is a single, integral system (Bernadskiy 1967).

The surface waters of rivers, lakes, reservoirs, seas and oceans receive tremendous quantities of various chemical compounds today, for which no precise accounting can be made. Apparently, there are several thousand such substances, and each year increasing numbers of substances are dumped, creating chemical pollution of the environment. The powerful inflow of pollutants changes the environment of aquatic organisms, as a result of which the quality of water decreases and the biologic productivity of commercial organisms is reduced. It is quite obvious that mankind cannot simply continue polluting his waters unchecked, but it is also impossible to exclude reservoirs and streams from the circle of human economic activity. The only proper path for establishment of the interrelationship of society with nature is efficient utilization of nature, designed to continue over many years. We must not simply protect or simply utilize without control the waters of surface reservoirs and streams, but rather we must utilize them efficiently and in a multiple use fashion, i.e., by many water users. In connection with these new tasks, the need arises to develop principles for estimation of water quality in reservoirs and evaluation of their normal state.

All reservoirs and streams undergo changes over a period of years in accordance with changes in climate, geologic-geographic variation and other changes, not related to the effects of human factors. Therefore, we must develop criteria which can be used to maintain reservoirs and streams in a state satisfying the needs of man. If man is not considered, any body of water is in its normal state, i.e., it corresponds to the surrounding conditions. Only man, based on his own needs, makes an evaluation as to whether the reservoir is in a normal or pathologic state. The time has come for regulated interrelationships between human society and nature. The need has arisen to develop principles and standards for estimating the quality of reservoirs, establishing limits of permissible changes in water quality and, finally, formulating requirements for man - that which he must not do with natural water.

Noted elsewhere (Stroganov 1977), in a work on the concepts of the norm and pathology in water toxicology, is a new approach to the solution of the problem at hand. Hydrobiologists cannot limit themselves to a simple description of what occurs in a reservoir following chemical pollution. An "engineering" method of thinking is required, i.e., we must first formulate how the body of water should be, then how this end can be achieved.

In order to formulate how a body of water should be, we must select principles, in accordance with which we can develop the necessary water quality indexes.

Based on the historic relationships between the abiotic medium of reservoirs and the hydrobiologic processes occurring in them, to which man has now been added, several principles can be formulated. These principles must lie at the base of the development of standards regulating the quality of water in reservoirs. It seems that theoretical problems of water toxicology should be solved in the aspect of development of principles.

In estimating the qualitative state of a reservoir, one can obtain varying answers, depending on our requirements, i.e., the initial standpoint. Among the many water users, the highest demands for water quality are those of but two: fishermen and those who drink the water. Therefore, all of the questions which are stated can be answered in terms of satisfaction in the reservoir of the condition of high productivity of commercial species and good quality of drinking water. If these standards are met, we must call this body of water a normal one; if they are not met, it must be considered an anomalous or even pathologic body of water. This last term is used by hydrobiologists, although it is not really quite applicable to bodies of water.

As the economy becomes increasingly industrialized and "chemicalized", a situation arises in which the need for fresh water of good quality increases greatly, both for various branches of the economy and for water supply for the population. However, the quality of fresh water is continually reduced, a situation which has led to great difficulties in water supply.

The Soviet Union has tremendous reserves of fresh water, but their distribution does not correspond to the needs of the regions with the greatest concentration of industrial entities, agriculture and other branches of the economy. Redistribution of fresh water over the territory of the country is quite expensive, and furthermore has great effects on the ecology of large areas. Therefore, various steps must be taken to preserve good quality of fresh water (purification of industrial wastes, improvement of the technology of production in order to decrease the consumption of water and dumping of wastewater into reservoirs, transition to closed cycles and dry technologies). In order to preserve the water quality which is needed, it is necessary to first of all limit the discharge of pollutants into reservoirs, i.e., standardize or regulate the discharge of chemical pollutants.

Various indexes characterize the level of pollution in water: chemical, bacteriologic, hydrobiologic and the MPC's for individual toxins. The chemical and biologic factors are the most widely used, the MPC's being less frequently used and hydrobiologic indexes being quite rarely used. However, it is hydrobiologic processes in reservoirs which play the decisive role in the formation of water quality. Aquatic organisms, on the one hand, develop their vital activity on the basis of hydrochemical and hydrologic modes; water for their habitation and, on the other hand, the predominance of various species of aquatic organisms determines the direction of hydrobiologic processes and thereby determines the nature of formation of water quality.

This interrelationship of water quality and hydrobiologic processes in a reservoir causes definite difficulties in standardization of the discharge of chemical pollutants into reservoirs and in the production of water quality. The necessity has arisen for indicating hydrologic principles which must form the basis for development of standards for the protection of good water quality, and for estimation of normal and pathologic states of reservoirs. To do this, let us discuss the main elements of the problem, in order to note paths for their solution.

In each reservoir, the quality of water is formed by all aquatic organisms. They pass through their bodies the entire mass of water of the reservoir, enriching it by many products of their metabolism and, simultaneously, changing the gas and mineral composition of the water. In the cycle of matter, some organisms play a determining role while others play a subordinate or even hardly noticeable role. Bacteria, protozoa, algae, and all invertebrate animals - the filter feeders - play a significant role.

A reservoir is a multicomponent system, consisting of living organisms and the water itself, containing various chemical substances in the molecular and supermolecular states, as well as the bottom, which contains a number of organisms and silt particles. The number of species is usually several hundred or even thousands in such reservoirs as Lake Baikal, while the number of individual substances is not precisely known, but it must be assumed that there are also several hundreds, or perhaps even thousands. For example, some of the large rivers pick up along their way not only several hundreds of different chemical compounds and ions, depending on the geochemical status of the watershed, but also several hundreds of chemical compounds from industrial enterprises, cities and population centers, water transport, and atmospheric precipitation. The complete chemical composition of such waters is unknown. We know indirectly that it includes a long list of substances.

This tremendous number of components in the water system is in total interaction and interrelation. The quality of water is a resultant of these many interrelationships. It is practically impossible to consider them all at the present time. Therefore, we must distinguish the most important determining components. This approach to determination of the regularities of behavior of an aquatic system is simplified, but is necessary in order to solve the problems of standardization of water quality which have been set before us.

Among aquatic organisms, three main functional groups must be distinguished: 1) producers - organisms which create organic matter in their bodies by the process of photosynthesis, utilizing mineral substances dissolved in the water (salts and gases); 2) consumers, transformers - organisms which construct their bodies by consuming organisms of group 1. This group includes phytophages and organisms which feed on the phytophages, i.e., predators; and 3) reducers. A large group of organisms (bacteria, protozoa, fungi) decompose the waste substances from the vital activity of other organisms as well as dead organisms, to mineral substances once more.

In each of these groups there are many species which follow each other in a regular sequence during the seasons of the year. The specific composition of each functional group changes depending on the specifics of the reservoir, its geographic position, climate, nature of bottom, hydrologic and hydrochemical modes. For the full cycle of matter in the reservoir, the specific composition of the functional groups (1-3) is of no great significance, while for commercial organisms (their nutrition, growth, breeding), the specific composition, particularly of organisms of the first group, may be of decisive significance. For direct consumption by man (commercial organisms), some organisms of the second functional group are of great significance.

Of the many hydrochemical components, substances defining the overall characteristics of the water (carbonate system, relationship of calcium and magnesium, sodium and calcium, chlorine and sulfate), as well as dissolved organic matter and biogenic elements (nitrogen, phosphorus, iron) and microelements (manganese, boron, copper, cobalt, etc.) are quite significant. To this normal composition of natural water, we must now add chemical pollutants, consisting of many different compounds, the chemical nature and biologic activity of which are not fully known. We do not know in what form they are present in the water and what are the paths of their transformation. We note that they always influence hydrobiologic processes in the reservoir. As a rule, this influence is not desirable for man and his activity. The aquatic organisms of each functional group have differing sensitivities to the effects of toxic substances which, with pollution, leads to restructuring of the specific composition within each group and among species from various groups. Toxic substances, depending on their chemical nature and concentration, suppress and reduce the population of some species while others are stimulated and increase their numbers, while still others are indifferent, i.e., retain their previous status (Stroganov 1978).

A change of dominance (predominant species) may not change the quantitative aspect of a functional group. It will play its role in the cycle of matter in a reservoir. However in the formation of good water quality and the creation of high productivity of commercial organisms, these changes in hydrobiologic processes may be undesirable. Therefore, we must limit the delivery of chemical pollutants to a body of water if we desire to use it for fishing purposes or for the supply of drinking water.

The interrelationships between functional groups in a reservoir can be drawn in the form of a diagram (Figure 1).

An actual body of water is an open system for both matter and energy. Therefore, reducers must process not only the substances which are transformed from primary organic matter by the producers, but also substances which enter the body of water from without. Usually, as organic matter in the water increases, the number of organisms which mineralize it also increases, but this process always involves some delay.

If we represent primary producers as P, all consumers and transformers as C and reducers as R, in the ideal case $P = C + R$. However, reducers cannot mineralize all dissolved organic matter completely, and some of it falls to the bottom sediment, while some remains in the dissolved state. Since there are sediments accumulated in past eras in all reservoirs, we can conclude that reducers have never been capable of mineralizing all of the dead organic matter in reservoirs. Consequently, the actual relationship has been: $P + A = C + R + O$, or $P + A = C + R + B + O$, where P is the primary organic matter of producers; A is that entering from without (allochthonic

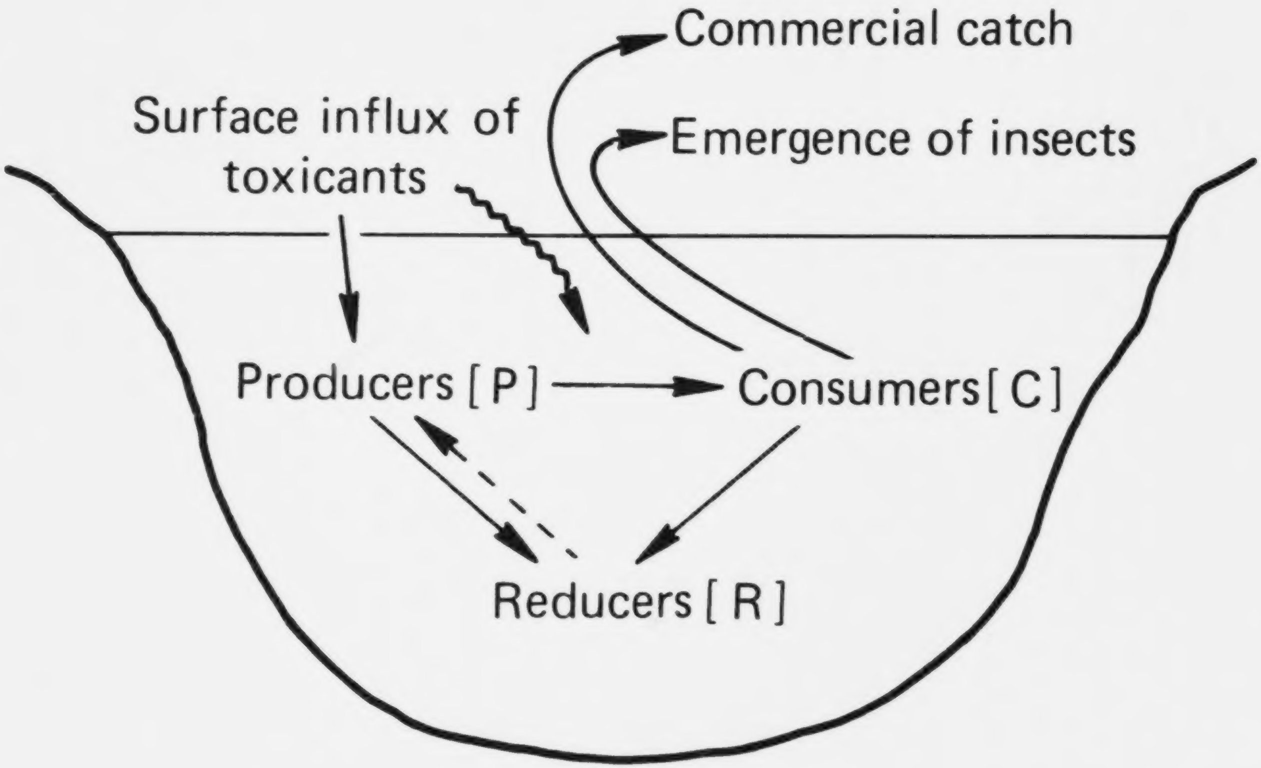
Commercial catch
Surface influx of toxicants
Emergence of insects
Producers [P]
Consumers [C]
Reducers [R]

Figure 1. Main functional groups in aquatic ecosystem.

matter); C is the organic matter in consumers; R is the organic matter in reducers and broken down by them; O represents bottom sediment and B is the catch of commercial species and insects which migrate out of the system.

At the present time, the situation is complicated by the fact that component A consists not only of organic matter washed away from the surface of the land, but also many toxic substances in industrial waste, residential sewage and flood water. If a reservoir is used for commercial purposes (fishing, catching of crabs and mollusks), some of the organic matter is removed from the reservoir in the form of commercial species. All industrial reservoirs are populated, particularly around their shores, with insect larvae, which leave the reservoir in the imago stage, thus carrying away a portion of the organic matter from the reservoir.

Chemical pollution acts on the entire aquatic ecosystem (living and indirect) and due to the variety in quality and sensitivity of living components of the system, restructures it in the direction of greater agreement to the new quality of the environment. This restructuring almost never satisfies the needs of humans. This is because processes of self-purification are suppressed. Reducers cannot process all of the matter polluting the water in such a short period of time. Water quality decreases and commercial species disappear.

Reducers function in a definite sequence (biologic oxidation, nitrification in two phases) and if the toxin breaks some link, the entire chain of processes of mineralization is broken.

We have studied the effects of many toxins of various chemical natures (metals, organometallic compounds, pesticides, antiseptics) and in all cases a common law is observed, as the concentration of the toxin increases, there is a delay in the development and an increase in the population of saprophytes and nitrifiers. The delay may be so long that self-purification is practically absent for 2-4 months. Figure 2 shows the variation of the several links of self-purification with concentration of toxins and time of action.

If this delay in mineralization processes occurs in a river, the polluted water flows downstream for 1000-1500 or more kilometers from the source of pollution. Quite naturally, the river carries traces of the effects of the chemical pollutant over this entire distance. Various filter feeders, particularly bivalve mollusks and Cladocera crustaceans, play a great role in processes of self-purification of water. However, they are sensitive to chemical pollution and their population drops quite rapidly, leading to a decrease in the self-purifying capability of the aquatic ecosystem with subsequent death of many species. The aquatic ecosystem is simplified to a small number of species and, if the chemical pollution continues to increase, the entire ecosystem may approach zero. This trend in aquatic communities is reported elsewhere (Stroganov 1978).

Of course, under today's conditions there are no surface natural bodies of water which have responded to pollution by complete death, but some small areas near industrial production facilities have approached this state.

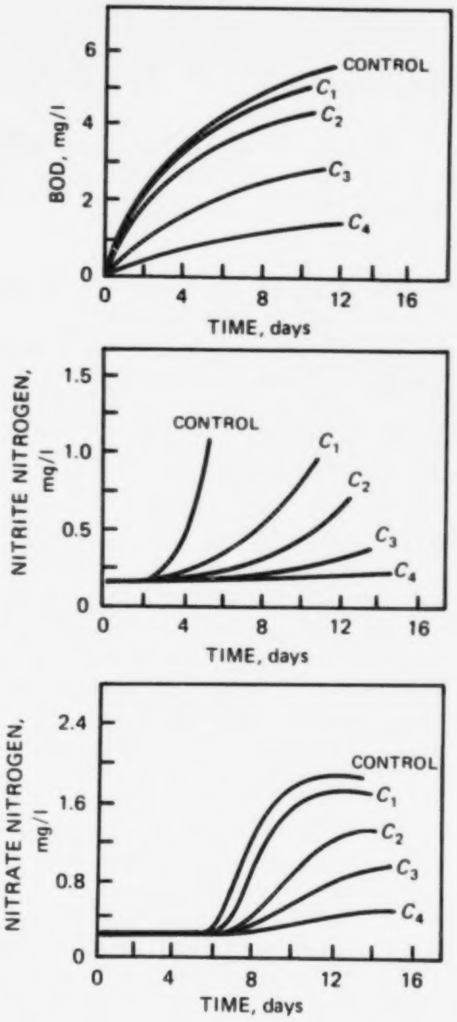

Figure 2. Summarized graphs of the main links in self-purification. Figures at the curves denote increasing concentrations.

Therefore, the entire picture of change is quite clear, the flora and fauna disappear.

The disappearance of valuable commercial species (which are usually sensitive to chemical pollution) has been described for some time in the literature. However, the scale of pollution and the variety of pollutants have increased greatly in the present century and particularly since the 1940s. Therefore, maintenance of reservoirs in a state desirable for man has become much more difficult.

We must see clearly that the struggle for pure water of good quality and containing valuable organisms is a difficult task, a long-term task requiring significant effort of the entire state and of intergovernmental organizations as well.

In terms of preservation of hydrobiologic processes in reservoirs, which assure the required quality of water and productivity of commercial species, we must limit the arrival of toxic substances into bodies of water. Of course, it would be quite good if we could completely eliminate any pollution (from the atmosphere, soil, waste and flood waters), but this is unrealistic, at least for the foreseeable future. Therefore, regulation and protection of reservoirs from toxic substances is a task of primary importance.

In developing specific indexes to be used to limit toxins, it is usually noted that, if a reservoir has a capacity for self-purification, it should be used, or allowed to purify all the discharge dumped into the reservoir. It is said that this is quite economical. This means of solution of the problem is quite favorable to the industry doing the polluting, but not to the nation, since other water users will be restricted or even denied the ability to use the polluted water. Our laws and constitution note that natural waters belong to the state and are used in a combined matter, i.e., by various water users.

Yet another suggestion has been heard to ease the burden on industry. Before waste waters are dumped into a reservoir, they should be diluted with pure water, thus accelerating self-purification of the water. Actually, as the concentration of organic substances and toxins decreases, the rate of self-purification increases. However, from where is this pure water to be taken for dilution at a time when the water consumption of industry is great and increasing rapidly? Furthermore, studies which we have performed show that the wastewaters of some chemical combines would have to be diluted by a factor of 200-500 to eliminate their toxicity (Stroganov, et al. 1978). There is not enough pure water for this purpose, and the water, which would be used, is not completely pure. Therefore, even the water in the deltas of large rivers is not completely pure, not completely suitable for drinking and fishing purposes. What is the answer?

The only effective answer to this problem is to decrease the quantity of toxins entering bodies of water. The achievements of science and technology, all technical progress, allow this to be done, but economic difficulties arise. The techniques needed to decrease the concentration on toxins in wastewater are expensive. No matter how expensive it may be, man must pay the price. The relationship between the cost of purification of water, the number of species of hydrobionts living in the water for a given level of pollution, and the degree of disruption of aquatic ecosystems can be expressed by the graphs of Figure 3.

A decrease in the purity of waste water (sewage and flood water, water polluted by water transportation, etc.) leads to a sharp decrease in the number of species; perhaps, first of all, a significant decrease in commercial species and, along with this, a significant increase in disruptions in the aquatic ecosystem. Money saved in reduced purification leads to money lost due to disruption of the normal (favorable for man) aquatic ecosystem.

Limitations of chemical pollution by means of the MPC significantly improve the situation, but do not guarantee complete safety. We must assume that: 1) the ecosystem includes more sensitive organisms than those which have been used in biologic testing to establish the MPC. Elimination of these species from the community may have an influence on the entire ecosystem. 2) Long-term after-effects may result from the influence of chemical pollutants on various vital processes of aquatic organisms. However, these two questions must now be stated as issues for the future. Even if all industrial enterprises, cities and large population centers purified their waste water to harmless concentrations for aquatic organisms, toxic substances would still reach reservoirs from the atmosphere and with water running off the surface of the land. We must assume that the body of water can handle this quantity of pollutant. If the self-purifying capacity of a body of water is somewhat greater than is currently being used, this excess amounts to a reserve of strength in the aquatic ecosystem. At the present time, many reservoirs cannot cope with the large quantities of chemical compounds entering them. They are functioning beyond the limits of the normal (useful for man) capacity of self-purification. As a result of this, any new addition of toxins to a body of water only increases the harmfulness of the water system for organims which are useful to man. As is shown in Figure 1, an aquatic ecosystem consists mainly of three functional groups of organisms, which perform vital processes at different rates. The rates are determined not only by the specifics of the organisms, but also by the environment (temperature, gas and salt composition and presence of toxins). Therefore, we must always consider that, for example, self-purification processes do not occur as rapidly as we would like, so that commercial species disappear. This disagreement between rates of self-purification and quantities of chemical pollution leads to long-term disruption of all hydrobiologic processes characteristic for pure reservoirs.

Based on the requirements of a reservoir in terms of preservation of hydrobiologic processes assuring pure water of good quality and productivity of valuable commercial species, the following four principles should be used as a basis for standardization of water quality in fresh surface bodies of water:

1. The principle of priority in the use of reservoirs. All large and medium sized reservoirs are used by many users, whose requirements for water quality vary greatly. The highest requirements for water quality are those

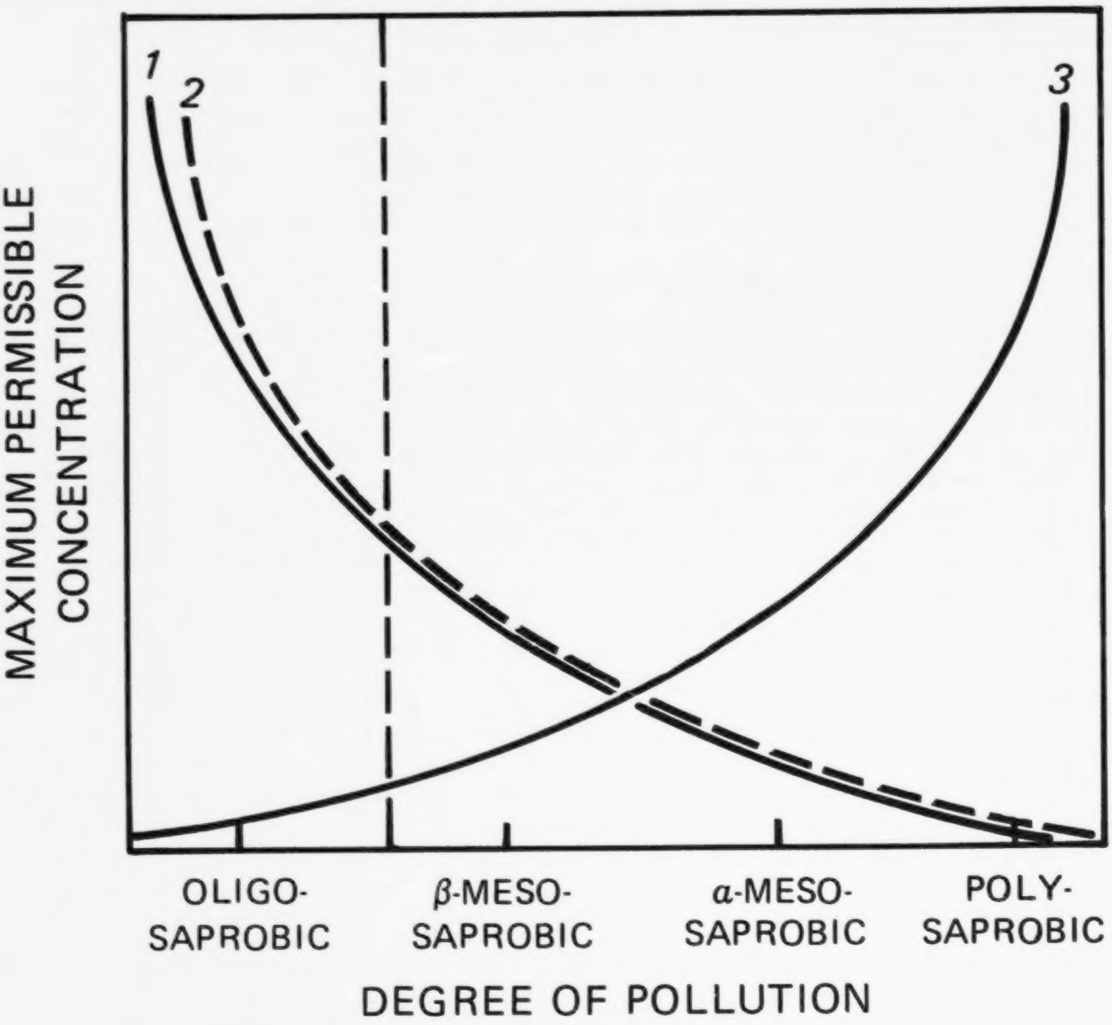

Figure 3. Relationship between degree of purification, pollution, number of species and disturbance of aquatic ecosystem. 1-Expenditures for treatment of waste waters, flood waters, and other pollutions; 2-Number of species in ecosystem; 3-Degree of disturbances in aquatic communities and ecosystems.

of fishing and drinking water supply. Only a few industries require water containing very low contents of salts. Such water users perform special water preparation measures on the water taken from the reservoir. Therefore, priority in the use of water is quite significant in the protection of water. In our water law it is noted that priority in the use of water must be given to organizations supplying water for drinking purposes and to fishing. Evaluations of the quality of the water and testing of water are performed by the Health Ministry and the Fishing Industry Ministry. This principle essentially lies at the basis of our water law, adopted in December 1970 (see sections 10, 15, 28, 31 and 37). Considering the great sensitivity of many species to chemical pollution, the formation of pure water of good quality by various species of aquatic organisms, and also considering the high sensitivity of valuable commercial species (fish, crabs, mollusks), priority should be given first of all to the fishing industry, with all of the results which follow from this (evaluation of water quality, testing and development of quality standards of discharge, etc.).

2. The principle of sufficient self-purification. This important principle is the basis of all subsequent principles. It means that all of the chemical pollutants which enter a reservoir must be mineralized to limits of concentration such that the species forming pure water of good quality and the species which are valuable commercial organisms can continue to exist. This means that for each region, climatic zone, the upper limit of self-purifying capacity of the water of a reservoir, which must not be exceeded, is the point of introduction of a greater quantity of pollutants than the body of water can process. Increasing the load of chemical pollution on a body of water above the limit of its self-purifying capacity leads to disruption of the principle of sufficient self-purification, leading to pollution of the body of water and degradation of the entire ecologic water system.

Processes of self-purification always occur (Figure 4), but not always with sufficient speed and completeness to assure the subsequent principles (i.e., 3 and 4). Therefore, self-purification may be sufficient for insensitive commercial species, but not sufficient for highly sensitive species and not sufficient to assure good quality of drinking water (principle 4). Consequently, the sufficiency of self-purification is evaluated on the basis of principle 1 (priority). For some water users, the requirements for water purity are lower and they may be satisfied with incomplete purification of water. Fishing and drinking water supply require water of the highest purity. Each water user can establish his own level of sufficiency of self-purification. We shall analyze it on the basis of the priority indicated earlier.

The quantitative indicators used to evaluate sufficient self-purification cannot be limited to BOD, COD AND O_2 content. Since we must always expect toxins to be present in water, we must determine the rate of processes of nitrification in both phases. As was noted earlier (Stroganov 1978), toxins decrease the rates of these processes, thus delaying the time of sufficient purification. In addition to these indexes, we must also have information on the toxicity of water for organisms. In most cases, nitrifying organisms are more sensitive to toxins than are saprophytes, while most

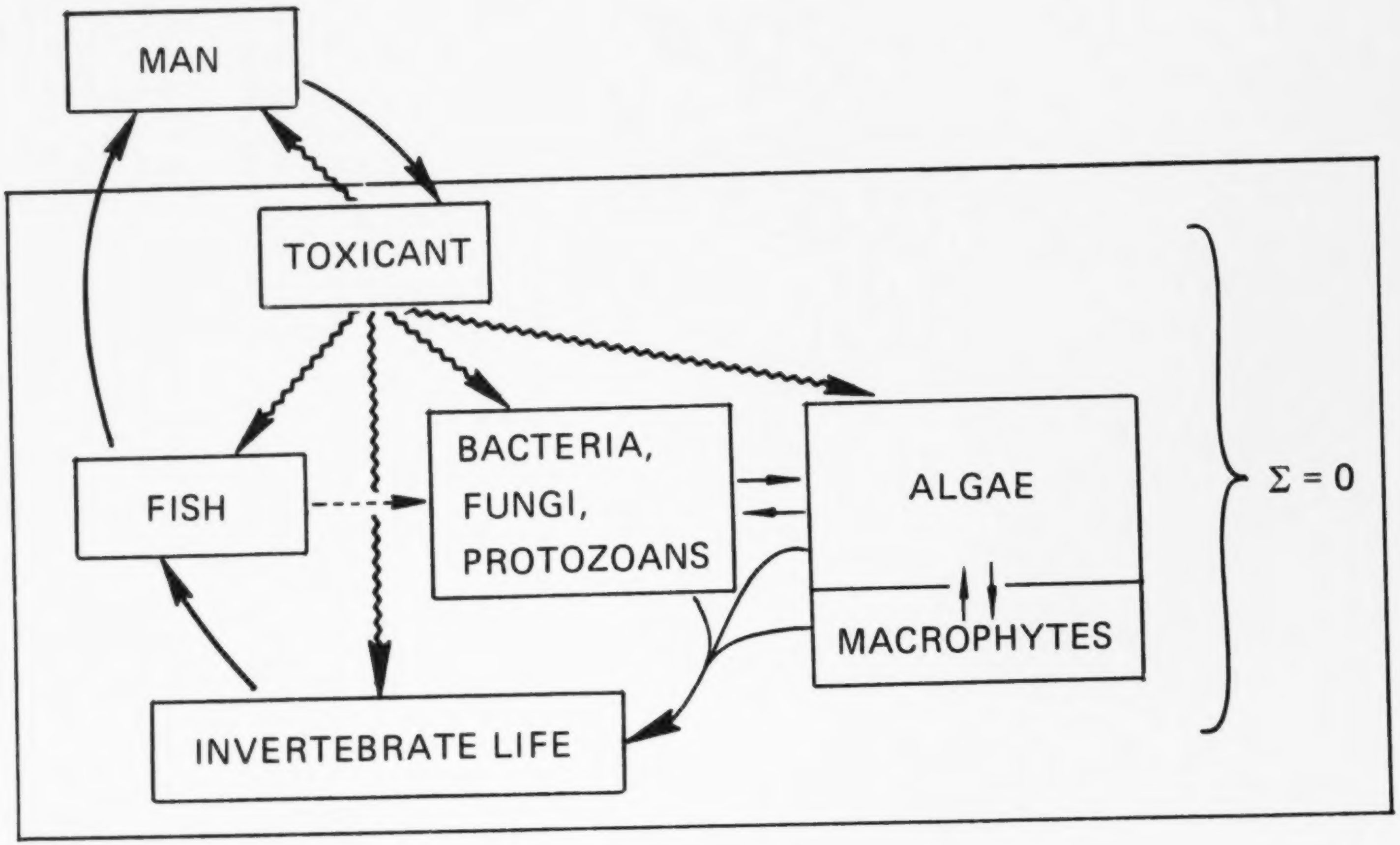
MAN
TOXICANT
FISH
BACTERIA,
FUNGI,
PROTOZOANS
ALGAE
MACROPHYTES
INVERTEBRATE LIFE
Σ = 0

Figure 4. Degradation of aquatic communities. Tendency to approach zero.

aquatic invertebrates and fish are still more sensitive than the nitrifiers. Therefore, we can evaluate water on the basis of the line of sufficient self-purification. More complex analysis, than is currently used, is required. We must also include toxicologic testing.

Certain toxic substances do not break down (e.g., metals) or break down poorly (some pesticides, detergents, etc.). In these cases, toxicologic testing will reveal their presence above impermissible concentrations. Chemical analysis is important and necessary for an overall description of the quality of water, but the indexes of self-purification and toxicity reflect another aspect, very important for the course of normal hydrobiologic processes.

3. The principle of assurance of conditions of life for commercial species. This principle falls entirely in the area of human evaluation. In addition to pure water of good quality, man also needs biologic resources found in reservoirs, particularly commercial species as a source of food and industrial raw materials. Valuable commercial organisms react sensitively to chemical pollution. They decrease their population or disappear as a result of death and migration to other water areas. Assurance of the conditions of life means the presence of water of a quality such that commercial species can continue to exist throughout their entire life cycle and do not lose their valuable qualities (growth rate, fertility, maintenance of high population, nonaccumulation of substances harmful to man, e.g., metals, pesticides, hydrocarbons, detergents, etc.). Chemical pollution may have both a direct effect on commercial organisms and an indirect effect through their food and the water in which they live.

It might be thought that, if the second principle is fulfilled, the third is not needed. However, the problem is more complex. Valuable commercial organisms and their sources of food are more sensitive than microorganisms participating in the decomposition of organic matter in water. Therefore, even if the second principle is fulfilled, though it is quite important, it is not sufficient to assure the third.

The qualitative and quantitative characteristics of this third principle are: the specific composition of commercial species, their population and biomass, ichthyofauna and the dimensions of the catch. Usually, the catch of aquatic organisms is the first sign of deterioration in water quality for commercial species, at a level at which the processes of self-purification reflect no danger.

At the present time, the importance of this principle is great, since the catch of aquatic organisms will become increasingly concentrated in inland bodies of water and the littoral waters of the oceans and seas in the near future. Hydrobiologic analysis encompasses essentially the entire ecologic system and catch and, therefore, most completely characterizes a given ecosystem with respect to its suitability for effective and complete utilization in the national economy.

4. The principle of suitability of water for drinking. The estimation of the suitability of water is usually performed by sanitary organizations.

We include this principle in hydrobiologic analysis because water quality is formed by aquatic organisms. What is the required quality of drinking water? In accordance with State Standard GOST 2874-73, water should be transparent, colorless and odorless, pleasant to taste, should contain no pathogenic organisms or toxic substances above the established MPC.

In analyzing water in accordance with the third principle, we find at times that water has long-term after-effects on aquatic organisms. They are manifested as changes in fertility, time of maturation, decreased dimensions of progeny and other deviations from characteristic parameters for the species. Determination of all of these problems forces medical and veterinary workers to ask the question of possible equivalent or similar influences on man and domestic animals using the same water for drinking purposes.

Summing up what we have said, it must be noted that evaluation of the quality of water in reservoirs from a broad hydrobiologic standpoint more reliably characterizes quality than other existing approaches. Chemical, physical and bacteriologic analyses cannot completely describe the quality of surface water today. The proposed hydrobiologic principles will help in developing a better scientific foundation for standardization of the quality of water of surface reservoirs. These principles are oriented toward developing standards for water quality in various regions and types of reservoirs used for fishing and drinking purposes.

The principles which we have set forth for estimation of normal and pathologic states of bodies of water suffering from chemical pollution are not new principles. They have been used and considered in the development of criteria for water quality. What is new is that the principles formulated are presented as a system for determination of the suitability (normality) or unsuitability (abnormality) of an aquatic ecosystem for the most demanding water users. These principles can serve as a basis for development of measures for standardization of water quality in reservoirs.

The principles formulated should assist in the development of standards for aquatic ecosystems based on the requirements of man's economic activity and life support. The criterion of the ecologic norm of a given reservoir might be the completeness with which the second, third and fourth principles are fulfilled. If these principles are excluded, evaluation of an aquatic ecosystem is senseless.

Under all conditions, man is the main standard for evaluation of the normality or abnormality of a body of water. The quality of water is becoming increasingly important for him. Therefore, evaluation of an aquatic ecosystem occurs primarily along the line of quality evaluation. It is not simply the number and variety of species, but rather useful species and their population and productivity; not simply the stability of the system, but rather the stability of the required quality of the system. Any ecosystem with time will reach stability given the surrounding conditions and becomes stable. An aquatic ecosystem is stable both with polysaprobic pollution, and with oligosaprobic pollution. In either case, it is stable, but the stability of the various qualities of water have different effects on man. Preference is given to the oligosaprobic state of a reservoir over the polysaprobic state. Aquatic organisms, as we know, are given preference in accordance with their physiology and biology. For them, a normal body of water is that which best corresponds to their physiologic and biologic peculiarities. A polysaprobic organism cannot live in pure water (oligosaprobic) and vice versa. Evaluation of what is normal in a reservoir can be performed by man, based on the principles outlined above.

Each organism also evaluates the quality of water in a reservoir. Can it live or not? Based on this evaluation, we can evaluate the usefulness of the ecosystem for man. Otherwise, we fall into unanswerable questions.

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SECTION 3

THEORETICAL ASPECTS OF THE "NORMALCY AND PATHOLOGY" PROBLEM IN AQUATIC ECOTOXICOLOGY

L.P. Braginsky[1]

During the rather short period of development of aquatic toxicology as a scientific trend, attention was mainly focused on the influence of toxicants upon selected aquatic organisms. The fundamentals of general toxicology established while investigating warm-blooded animals were the guiding principles in this research. Life, however is diverse and complex, and biology is multifaceted. That is the reason such an approach is insufficient. It does not include many of the consequences the influences of toxicants on the living matter of the hydrosphere.

In medicine and veterinary science, many variations from certain standard average values, characterizing vital manifestations and considered as "the norm", are usually defined by the concept "pathology". Continuing further with this analogy, medicine, veterinary science, phytopathology and ichthyopathology in solving particular problems of diagnosis and treatment of various human, animal and plant diseases, are based on general pathology, the disease theory. However, even in such a highly developed science as medicine, which for many centuries has accumulated information about human organism functioning, the concepts of "norms" or "standards" are highly indefinite. Only very recently has a special science related to healthy humans, normology, begun to develop in medicine. In both veterinary science and ichthyopathology this problem remains completely unsettled.

Our knowledge about the biological, physiological, and biochemical processes of aquatic organisms is so poor and insufficient, that in every separate case it is necessary to start a toxicological investigation from the study of the norm, and then to draw conclusions about various pathological effects as a result of studying the responses of known test-organisms to toxicants, while the number of aquatic species amount to hundreds of thousands, or even millions.

For these reasons, aquatic toxicology and data storage needs tend to define existing concepts of the normalcy and pathology of aquatic organisms under toxic environmental conditions. Recently, Soviet scientists have given much attention to this problem. However, as analysis of the present information has shown, primary attention is given to the analysis of normalcy and pathology at the organism and suborganism levels. Meanwhile, aquatic life specificity lies in the fact that aquatic organisms live in communities of different rank, and only their combined activity is of decisive importance in the formation of those aquatic ecosystem characteristics which are of interest to man, i.e., biological productivity and the maintenance of proper water quality.

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Mass biological processes are of considerable importance for understanding the processes of water quality formation. It is these processes which lead to community structure transformation and the disturbance of balance in ecosystems, i.e., the processes at the supra-organism level, which are objects of ecological/hydrobiological investigation, not the individual responses of organisms to a toxicant.

A new trend in ecology, ecotoxicology, which has been recently developed, and has already won world-wide recognition, deals not with the individual organism response to toxic effects, but with the response of the community and ecosystem, as well as the transformation of toxicants in natural ecosystems. That is why it is necessary to understand the concepts of normalcy and pathology at the supra-organism level of life organization. What is a normal population? What is a population in the state of "pathology"? What is a normal and a "pathological" biocenosis? What is a "normal" and "unhealthy" ecosystem? Finally, what is an "unhealthy" body of water, or "Krankenzee" described by German authors?

It is not easy to answer these questions, especially considering the extreme lack of knowledge of the consistencies of supra-organism system functioning. At the same time, it is clear that analysis of this problem cannot be guided by those initial concepts by which medicine, veterinary science and ichthyopathology operate, since the processes taking place at the supra-organism level are inadequate for the organism level processes.

In this report the question of "normalcy" and "pathology" of the supra-organism system is discussed from the points of view of demographic ecology and synecology.

POPULATION LEVEL

One of the major criteria of conditions favorable to populations is the ratio between birth and death. It is very difficult to consider this factor under natural conditions, but it may be characterized rather accurately in experiments with synchronized test-cultures of short lived invertebrates. In chronic toxicity tests with cultures of various Cladocera, after a series of 5-6 generations a decline in fecundity of females as well as offspring survival is observable. Similarly, an increase in mortality and a subsequent diminution of population can be noted.

One of the "pathology" indices at the population level, which can well estimate statistically and interpret graphically is the potential productivity value. This value is calculated by an equation, which connects the main biological parameters of the Cladocera, including lifetime of female, the number of litters during a lifetime, intervals between litters, juvenile numbers per litter, duration of maturation period duration prior to the first litter, with the value of potential population productivity (Pigaiko 1971). If potential population productivity is reduced from generation to generation, then it is a visual indicator of its pathological state, and the increase of potential productivity, or its maintenance at a stable state, are indicative of well-being, i.e., of the relative norm (Braginsky, et al. 1979).

Apparently, a number of biological productivity methods of assessment of aquatic animals, established for general hydrobiology (Vinberg 1968) with proper ecological and toxicological interpretation can be used in an analogous way to demonstrate the pathological state of a population of aquatic animals under toxic environment conditions.

For parthenogenetic invertebrates, i.e., Cladocera, Rotatoria, a switch to sexual reproduction and laying of subitan eggs (ephippia) indicate unfavorable conditions. However, under the influence of toxicants, this response is not always observed. Thus, the shift to sexual reproduction and formation of ephippia in Daphnia is absent in those cases exposed to chronic additions of low concentrations of phenyl urea derivatives, triazine, heavy metals, and surfactants. However, other pathological phenomena such as the appearance of dwarf males and parthenogenesis in specimens of half the size of the controls are observed.

The most frequent manifestation of pathological disturbances in Cladocera is egg abortion and the appearance of embryonic malformations. While these disturbances may be considered as a change at the organism level, their mass manifestation influences the fate of populations considerably.

Fluctuations in the number of aquatic populations in nature are highly diverse, and depend upon many factors for which it is difficult to account. Thus, knowledge of causes and mechanisms of these fluctuations is still extremely scanty. For this reason it is better to confine present activities to the concept of developing model laboratory investigations.

In the conduct of aquatic toxicological experiments it is necessary to resort to the study of laboratory "mini-populations" or "pseudo-populations". An elementary estimation of the median lethal concentration is made on the population model. If an experimental group of warm-blooded animals or fishes is impossible to consider as population, and the LC_{50} value obtained from invertebrates is interpreted as an individual mean, then the analogous group of invertebrate offspring are derived from the same parent and may be considered as an extract of a single population. As experience shows, conclusions drawn from studying such test-culture are in generally valid for aquatic ecosystems where the same species may be represented by a rather numerous population.

It is useful to consider the significance to the population the criterion LC_{50}. A wide utilization of this toxicometric criterion means that the death of the test-organism is recognized as the most authentic indicator of the toxic action of a substance.

It is a criterion which is beyond the concept of normalcy and pathology, since death represents a leap to a new quality to which no characterizable biological concepts can be applied. In this case the biological essence of death is disregarded, and the result of an experiment is considered as simply the answer to the question: is the substance toxic or not? But at the population level, the essence of this question is different. The LC_{50} criterion itself means that any population is heterogeneous in relation to its sensitivity to the toxicant. It suggests that there are resistant and tolerant individuals within it, and, therefore, the toxicant functions as a factor of natural selection with regard to the fate of the population.

Mortality as an ecological and evolutional factor controlling population numbers has appeared together with life, and it would disappear only together with it. If death means an awful and final defeat in the struggle for existence for an individual, then for a population mass death is only the elimination of the less adaptative, the survival of the more adaptative incorporates some form of "reorganization", the essence of which is that the population number declines abruptly first, then as resistant forms appear, a population numbers outbreak is observed. A health experience with insecticide application is evidence of this phenomena. As a result of wide utilization of strong insecticides, the insects not only survived but on the contrary reproduced intensively. Aquatic animals are no exception to this phenomena. It is known for instance, that mosquito fish resistant to DDT have recently appeared (Holden 1973). In another case, a Cladocera test-culture appeared to be killed in the V-VI-th generation under the influence of toxicants, however, the XY-XYI-th generation "suddenly" revived and began to breed rapidly. Finally, an algae culture almost killed under the influence of algaecide preparations was able to recover, and new cell generations grew. In principle, all these phenomena mean that the population has latent resources to aid in elimination, and with the decrease of environmental toxicant concentration, it can function as stimulative factor for reproduction of the organisms inhibited by it, in accordance with the law of phase reactions.

Aquatic organisms, in contrast to warm-blooded animals, have other latent resources, namely the ability to survive unfavorable conditions in a resting stage, i.e., the statoblasts of moss animals, turions of aquatic animals, ephippia of Cladocera, spores and cysts of Protozoa, the closing of mollusk, shells, and the resting stage of algae. All these forms of life exist in sediments, and are not susceptible to toxic effects. The adaptation to very severe conditions in water is a rather good protection against toxic agents, and it serves to guard populations from destruction by toxic substances. In contrast to poikilothermal aquatic species, homothermal organisms are physiologically only accessible to poisons under conditions of optimal temperature. At temperatures below 15°C, their biologic processes are so inhibited, and exchange with environment is so reduced the the presence of a toxicant in their environment is of no serious danger to them. Thus, the toxicity of a substance, and the even higher values of the LC_{50} obtained in the experiments with actively functioning individuals is not necessarily evidence of its danger to a population. These factors serve only to warn about toxic effects under conditions of optimal temperature. When the temperature of water is raised to 30°C, the toxicity of a given substance for organisms can be increased by hundreds, thousands, and tens of thousands times. This has been demonstrated in experiments with cadmium on Daphnia magna (Braginsky and Scherban 1978). Therefore, the question of the "pathological" reactions of aquatic populations to toxic effects is inseparably linked with ambient temperatures.

The existence of populations, as opposed to individuals, is in itself protective, since an irregular distribution of a toxic agent within population predetermines the possibility of preserving some quantity of resistant individuals. This was noted in natural communities of the blue-green algae treated with algaecide preparations. Luminiscence microscopy data showed that from 0.5 to 20 percent of the total quantity of algae was unaffected by algaecides. In experiments with aquatic invertebrates, uneven mortality of test organisms was observed, although it was not possible to connect this phenomenon directly with the level of toxicant accumulation in the animals' body.

An irregularity of toxicant distribution among fish populations was confirmed analytically by gas chromotography for extracts of DDT in organs and tissues. When studying accumulation levels of this pesticide in fish populations, fluctuations in cerebral fat tissue from 0 to 40 mg/kg were observed, consistent with a normal distribution range. It is natural that fish with DDT levels exceeding the critical values (3 mg/kg of cerebrum weight) are in a state of deep pathology; a cumulative intoxication which does not affect the entire population (Braginsky, et al. 1979).

All analogeous phenomena are undoubtedly similar, and subject to the law of survival of the species since the history of the earth, toxic factors are not new. They probably functioned constantly in the early stages of the development of the planet, with respect to high concentrations of ammonia, methane, phosphorus and other toxic agents in water. The "chemical weapon" is of importance to interspecies relations, and where this weapon was used, protective measures were created. Apparently these measures are also effective with respect to toxicants of anthropogenic origin. Whatever the mechanism is for populations reaction to toxic effects, the ultimate result should be a decrease of population abundance. Occasionally, the population may even increase, when concentrations promoting reproduction are favored. In any case, the question where "normalcy" ends and "pathology" begins is a controversial consideration. It must be noted that deceleration or acceleration of a population's reproduction rate, or fluctuations in its range of abundance are not something fatal or unfamiliar. Sequential sigmoid fluctuations of population quantity are characteristic of life on earth; therefore, it is hardly appropriate to speak about pathology in the same sense in which the term is used in medicine.

THE LEVEL OF THE COMMUNITY AND THE ECOSYSTEM

The most greatest problem of the present, the problem of clean water, is connected not with the processes of individual and population levels, but with the synecological processes, since water quality is a function of the combined living activity of aquatic organisms. Therefore, the final criteria in assessing toxicant effects on an aquatic population as a whole, i.e., criteria of "normalcy" and "pathology", are the processes taking place within complex biological formations, the community and the ecosystems.

Toxicant inputs into a natural ecosystem leads to a rather specific situation, the major features of which may be characterized as follows:

1. The toxicant is directed not towards a single target organism as it is under experimental conditions in aquarium, or in the whole in vitro system, where the isolated "toxicant-organism" relationship is artificially created, but rather, the toxicant effects on variety of targets;

2. As a result of spectrum of action, its concentration is dispersed and the real dose per organism is not equivalent to the present projected concentration;

3. The toxicant quantity per biological organism depends on population density, biomass, species diversity, the presence of the most susceptible organisms consuming the given toxicant, and on many other factors;

4. Immediately after entering an ecosystem, the toxicant is attacked by active lower organisms, begins to undergo biodegradation by various exoenzymes, and is intercepted by species susceptible to accumulation;

5. A decrease in concentration as a result of the process of detoxication, dispersion, physico-chemical destruction, and sorbtion of the toxicant promotes phase reactions, which may be responsible for both inhibition and stimulation of vital activity of aquatic organisms.

Thus in an aquatic ecosystem, the toxicant encounters the system functioning as a whole: it is a negatively eutropic system, and the toxicant is an entropic factor destroying life. Between the entropic factor, and the system inclined toward negative eutrophy, a struggle starts. In the system a counteraction grows in an effort to destroy the entropic factor. This creates its specific quality buffering, described in the works of M.M. Kamshilov (1973). The system consumes and transforms the toxicant, but only within certain limits. When this potential of resistance is exhausted, a toxic effect is manifested.

Because of this situation, bodies of water with varying trophic status have varying degrees of resistance to toxicants, and varying rates of transition to the state of disturbed balance. Generally, the richer in life a body of water, and the more diverse this life quantitatively and qualitatively, the slower is the transition from normalcy to pathology. This suggests that eutrophic systems should be less liable to the effects of toxic substances than oligotrophic and distrophic ones. In this connection the unstudied problems of toxicity criteria (normalcy and pathology) at the sapra-organism level of life organization arise. The difficulty of their formulation lies in the fact that the scientific fundamentals of functional community studies are not established, and the present knowledge of community structure is mainly the knowledge of morphology, composition, quantity, biomass, occurrence, and various indices or relationship between the major components in the structure. It concerns planktonic as well as bottom communities, and also the other less studied group of aquatic animals.

Nevertheless, even the morphological approach and related experimental investigations permits discovery of some of the specific features of community reactions to toxic effects. To understand these reactions, it is necessary to use the concepts of dominant, subdominant, and "shelf" forms. The results of ecological investigations show that in ecosystems not influenced extensively by man, the structure of communities and the character of seasonal changes are rather stable, and may be of the same type over a period of many years. In waters polluted by toxic substances, or in ecosystems under conditions of experimental influence, characteristic features become visible, including a shift of the dominant forms. Occasionally, shifts are very abrupt and conditioned by the fact that the dominate forms are inhibited or eliminated completely, whereas forms of minor importance reach the maximum of abundance and biomass (Braginsky 1975; Braginsky, et al. 1979). The shift of other community components may be observed, and these changes occur spasmodically as well as slowly in accordance with the degree of toxic effect, toxicant concentration, selectivity of action, community specific composition, and many other factors. Moreover, there is a change in total numbers, and in biomass of organisms, as well as an exchange of roles in the structural components of biocenosis, i.e., a change in hierarchical relationships. Under the influence of very strong toxicants, the community may be completely destroyed, and then the system becomes nonstructural. Apparently, the latter may be considered as an indicator of obvious pathology, whereas the shift of dominant forms is not a pathological process, but represents a form of community stabilization under new conditions. The second case is the typical manifestation of degradation, the mechanism of which has been studied in detail (Stroganov 1974).

Experimental investigations and mathematical modeling had demonstrated that aquatic communities, generally speaking, may exist in three stable states: 1) initial, 2) functionally and structurally reversibly altered, and 3) irreversibly altered. The second level of change is characterized as ecological fluctuation, the third as a shift of dominant forms. These do not represent pathology, but simply the normal range of community variability related to adaptational changes. Apparently, "pathology" begins when the system passed the third level of stability and approaches the nonstructural level. In mathematical models this process is shown by a parabola and indicates the approach of ecological catastrophe.

The structure, i.e., regularity, is characterized by the presence of a reserve of negative entropy. "Destructuring" indicates the development of processes of entropy, a movement in the direction of "chaos" (Hilmy 1968). This is a physical indication of the process promoted by the influence of toxicants in ecosystem. However, as it was previously noted, the system as a whole is a complex of factors, among which microorganisms and Protozoa play a chief role to counteract entropy (Kamshilov 1973; Braginsky 1975; Geptner 1977). The toxicant is "dispersed" in ecosystem and under the influence of microorganisms its concentration decreases. In the end, it determines ecosystem buffering, its ability to consume and transform a certain quantity of toxicant (Kamshilov 1973).

Buffering may be considered the degree of negative entropy of the system as a major factor of preservation of its normal life. The transition to "pathology" begins when the buffering limit is reached, and the system is unable to withstand this toxic effect.

Now we approach the main question of the problem of clean water: what is a "pathological" waterbody or ecosystem, and how does it differ from a "normal" one? In the light of the previous discussion, it appears as if the answer should be: an ecosystem in a "pathological" state is a body of water with a disturbed buffer system, in which the detoxification potential is suppressed and negative entropy processes yield to the entropic processes, i.e., degradational ones.

One of the manifestations of such a state is an increased mortality within community populations, particularly among highly organized life forms; differing, as a rule, by a greater tolerance to toxicants. As a result of the increased death rate, population dynamics, age and sex ratio changes, community structure changes correspondingly, and the system shifts to a qualitatively different state. This state may be rather stable, particularly if the population which is resistant to toxicants becomes predominant, or unstable, with the tendency to further degradation, if this population also is rather tolerant to toxicants. In certain individuals (as in the intermediate stage between the normal state and death) various pathological disturbances appear, which may be considered indicative of unfavorable conditions in the system. Symptoms may include disturbances in enzyme systems and other biochemical changes corresponding functional disturbances, structural pathohistological changes, alterations of conditioned reflex activity, and behavioral reactions studied by toxicologists on the organism and suborganism levels.

Recently, it is difficult to tell what relationship exists between disturbance of various functions and the structure of some organisms, including fish. Of particular concern are the lethal concentrations of toxicants and their threat to aquatic life at supra-organism levels. Critical, then, is the extent that clear and evident pathological changes at organism level reflect the "pathology" of supra-organism level, i.e., the community or the ecosystem, since every lower level of organization is less resistant to toxic factors than the next higher one, and the ecosystem is in danger of catastrophe only when all of the buffer systems at lower levels are destroyed.

The notions of normal and pathological states of aquatic ecosystems are closely associated with the whole complex of other ecological concepts such as preservation of homeostasis, transformation of community structure, a shift of dominant forms, disturbances of bio-geochemical cycles, system buffering, detoxification potential and, finally, with the concept of entropy and negative entropy system.

From this point of view we consider the study of the general problems of pathology of aquatic ecosystems in the light of the second principle of thermodynamics. The consideration of the problem of detoxification of waters should then be from the view of life as a negatively entropic process, evoked by our planet to retain energy, and to prevent its dispersion into space.

In the same way that consideration of the flux of substances and energy in aquatic systems from a position of the law of conservation of energy promoted fruitful solution of many problems in productional hydrobiology, the analysis of aquatic ecosystem responses to toxicants effects in the light of the second principle of thermodynamics may significantly stimulate our understanding of the destructive and reduction processes and factors, determining the stability and degradation of aquatic ecosystems, and the hydrobiosphere as a whole.

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SECTION 4

TRENDS IN AQUATIC TOXICOLOGY IN THE UNITED STATES: A PERSPECTIVE

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The need for toxicology testing has increased during the 1970's. It was expanded for pesticide registration; many of the same requirements for pesticide registration will be required for toxic substances approval; and acute and some chronic toxicity testing are being required for ocean dumping permits. Research approaches are changing from acute toxicity testing and residue analysis to more complex and integrated research involving chronic toxicity, clinical chemistry, and ecosystem concepts. These approaches are resulting in assessments of the environmental hazard of contaminants, sometimes even before they enter the environment, rather than in the production of acute toxicity and residue data of only limited value. Also, the integrated approach is providing basic scientific concepts that are essential in the prediction of environmental hazards.

Developmental research is providing better interpretation and shortcuts in toxicology. In ecosystem studies, scientists are determining what really must be measured to assess the type and degree of pollution; biochemical techniques are decreasing the time required for chronic toxicity studies; and organisms other than fish (plants and invertebrates) are being recognized for their importance to fish and aquatic ecosystems and are being tested accordingly. Recognition of the complexity of aquatic contaminant residues has led to increased emphasis on the development of integrated strategies for their detection and analysis.

Research emphasis has shifted from the problems of persistent organochlorine pesticides to the prediction of problems that may arise as mining, smelting, and coal conversion are increased, new methods of sewage disposal, petroleum and detergent use expands, and pesticides use changes in forest, range, and agricultural practices. The increasing concern of industry with environmental problems is resulting in joint industry-government research, not only to assess hazards, but to further define less hazardous substitutes. A new interest is emerging in metals and other inorganics. Although the literature contains abundant research on organics, much of it is unusable, and it is difficult to predict the environmental impact of energy development and the associated inorganic contaminants. There is a rapidly increasing trend toward use of larger quantities and greater varieties of herbicides in agriculture. New forest management techniques call for control of scrub and hardwood vegetation over vast acreages; no-till farming practices require greater uses of herbicides and herbicide mixtures; and conversion of riparian vegetation into agricultural uses results in herbicide and insecticide run-off. All of the problems with persistent organochlorine pesticides are not gone, however. Decisions concerning some of them still await a stronger factual base; others merely require monitoring and surveillance to pinpoint problem areas and insure that the residue trends continue downward.

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Specific research advances and developments in aquatic toxicology in the United States are presented here.

TOXICITY TESTING

Acute Toxicity

Toxicologists are well aware of the virtues and limitations of the acute toxicity measure; yet, there are probably few measurements that have been as misunderstood in evaluating hazard or safety of a chemical to aquatic life as the LC50 (concentration lethal to 50 percent of the organisms within a given period--usually $\leq$96 h). Users of any acute toxicity data must bear in mind that the LC50 measures only one biological response -- a lethal one. Its main value is to provide a relative starting point for the evaluation, along with other measurements (e.g., water solubility of the chemical, its partition coefficient, its degradation rate), of environmental hazard. In addition, the acute toxicity test provides a rapid, cost efficient way to measure relative toxicity of different forms and formulations of a chemical, its toxicity in different types of water (acidic, basic, hard, cold, warm), and its toxicity to organisms representing different trophic levels. Until other techniques can be shown to be equal or more meaningful to aquatic toxicologists, the acute toxicity test is here to stay.

Chronic Toxicity

Partial and complete life-cycle toxicity tests with fish have become commonplace, and provide data on survival, growth, reproduction, and other sublethal responses. However, these tests can be expensive, high-risk investigations that may require up to a year to conduct. Recent evaluations (Eaton 1974; Macek and Sleight 1977; McKim 1977) have shown that 30- to 60-day toxicity tests on embryos and larvae may provide data as sensitive as that observed in partial and complete life-cycle tests. The maximum acceptable toxicant concentrations (MATC) derived from tests with embryos and larvae, or juveniles were usually equal to, but never exceeded a factor of 3 times the MATC values derived with partial or complete life-cyle tests (Table 1).

TABLE 1. MAXIMUM ACCEPTABLE TOXICANT CONCENTRATIONS (MATC) FROM PARTIAL AND COMPLETE LIFE-CYCLE TOXICITY TESTS WITH FISH AS COMPARED WITH MATC'S DERIVED FROM EMBRYO, LARVAE, AND EARLY JUVENILE TOXICITY TESTS[1]

Toxicant	Fish Species	Partial/complete life-cycle MATCs (μg/l)	Embryo-larval/ juvenile MATCs (μg/l)
Pesticides			
Acrolein	Fathead minnow	11 - 42	11 - 42
Atrazine	Brook trout	60 - 120	120 - 240
Trifluralin	Fathead minnow	2.0 - 5.1	5.1 - 8.2
Endosulfan	Fathead minnow	0.20 - 0.40	0.20 - 0.40
Endrin	Flagfish	0.22 - 0.30	0.22 - 0.30
Heptachlor	Fathead minnow	0.86 - 1.8	0.86 - 1.8
Diazinon	Flagfish	54 - 88	54 - 88
	Fathead minnow	6.8 - 14	6.8 - 14
Guthion	Fathead minnow	0.33 - 0.51	0.70 - 1.8
Malathion	Flagfish	8.6 - 11	8.6 - 11
PCBs			
Aroclor 1242	Fathead minnow	5.4 - 15	5.4 - 15
Aroclor 1248	Fathead minnow	1.1 - 3.0	1.1 - 4.4
Aroclor 1254	Fathead minnow	1.8 - 4.6	1.8 - 4.6
Aroclor 1260	Fathead minnow	2.1 - 4.0	2.1 - 4.0
Metals			
Cadmium	Flagfish	4.1 - 8.1	8.1 - 16
	Fathead minnow	37 - 57	37 - 57
Chromium	Fathead minnow	1,000 - 3,950	1,000 - 3,950
Copper	Brook trout	9.5 - 17	9.5 - 17
	Fathead minnow	11 - 18	11 - 18
Lead	Brook trout	58 - 119	58 - 119
	Flagfish	31 - 62	62 - 125
Nickel	Fathead minnow	380 - 730	380 - 730
Zinc	Flagfish	26 - 51	51 - 85
	Fathead minnow	30 - 180	30 - 180

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[1] Condensed from McKim (1977).

Other research being conducted that involves short-cut methods to chronic toxicity studies has been highlighted by the U.S. Environmental Protection Agency's Environmental Research Laboratory-Duluth (1977-1979) and includes the following advances:

1. Measurement of ventilatory patterns of fish with a microcomputer monitoring system.
2. Use of fish cough frequency as an estimate of chronic toxicity.
3. Development of a rapid toxicity test in which the fingernail clam is used.
4. Monitoring liver aryl hydrocarbon hydroxylase induction in fish.
5. Changes in steroid hormone metabolism in fish.
6. Saltwater tolerance and smoltification in salmon.

Aquatic Plants

The effect of point and non-point source contaminants on submersed rooted vegetation is little known. The contribution of submersed rooted aquatic macrophytes to the ecological support of fishery and wildlife resources can be separated into three general categories:

1. Numerous species of mammals and waterfowl are directly dependent on macrophytes as food. For example, the stems, leaves, seeds, and rootstock of sago pondweed constitute up to 50 percent of the diet of migratory ducks and geese. Submersed rooted macrophytes are also required by fish for forage, cover, and spawning; furthermore, they provide an important substratum for invertebrates eaten by fish.
2. The overall metabolism of aquatic systems (lakes and streams) supporting fisheries is dependent to a major extent on the detritus components of dead, dissolved, and particulate organic carbon which form the primary source of biological energy. Beds of submersed, littoral, rooted macrophytes contribute a large part of the organic detritus in all but a few aquatic systems.
3. Littoral vegetation also modulates the flow of inorganic nutrients from the watershed to the limnetic area and stabilizes and controls the magnitude of planktonic photosynthesis in lakes.

In addition, contaminants deposited in bottom muds may be taken up by plants and passed along a detrital food chain, ultimately to fish, waterfowl, and other organisms closely associated with aquatic ecosystems. To estimate the effects of contaminants on rooted aquatic vegetation, we are examining the following variables for inclusion in chronic laboratory tests with appropriate species: growth, reproduction, photosynthesis, nutritive value, and residues. The transfer of residues through food chains of which the exposed vegetation is a part is also being investigated

CLINICAL (DIAGNOSTIC) TESTS

The use of diagnostic tests in hazard assessment procedures can decrease the time required for safety evaluation of chemicals, define no-effect exposure concentrations more adequately, and provide a better understanding of the mode of action of chemicals. Routine diagnostic tests are frequently not available to aquatic toxicologists because biochemical and physiological research has been minimal in aquatic toxicology, which is a relatively new field of science, as compared to such fields as human medicine (Mehrle and Mayer 1979). The "state of the art" of physiological, biochemical, and histological tests in aquatic toxicology held at Pellston, Michigan (Macek et al. 1978). The participants rated the relative utility of eleven toxicity tests, using the criteria of ecological significance of effects, scientific and legal defensibility, availability of acceptable methods, utility of test results in predicting effects in aquatic environments, the general applicability to all classes of chemicals, and the simplicity and cost of the test. In terms of present utility for use in assessing the hazard to aquatic environments, acute lethality tests were rated highest, followed by embryo-larval tests, chronic toxicity tests measuring reproductive effects, and residue accumulation studies. Histological tests ranked ninth, and physiological and biochemical tests tenth in overall and present relative utility because of the inability to relate the results of these tests to adverse environmental impacts.

Physiological and biochemical tests are generally not conducted for two reasons: (1) it is felt that they are mainly useful in evaluating the mode of action of chemicals (Brungs and Mount 1978); or (2) there is not enough basic information known about fish physiology and biochemistry to ascertain the ultimate effects, since alterations in these processes do not necessarily indicate a disadvantage to the survival and success of the organisms.

The analytical techniques and instrumentation are well developed for performing clinical analyses, and considerable research on physiological and biochemical responses induced by chemical toxicants has been conducted, but useful biological or diagnostic indicators have not been developed. In our opinion, the main reason for this lack of progress has been the lack of a comprehensive, integrated approach in toxicological studies with fish. To overcome this problem, researchers must conduct biochemical, physiological, and histopathological investigations in conjunction with toxicity studies that measure important whole-animal responses. Establishing the relationship of organism to sub-organism responses will help insure development of pertinent diagnostic indicators of fish health. The choice of whole-animal responses to evaluate in toxicity studies with fish depends on the purpose of the toxicology program, but in most aquatic toxicology programs, emphasis is given to toxicant effects on survival, growth and development, reproduction, and adaptability.

To adequately assess the influence of contaminants on the aquatic environment and to overcome the avoidance of biochemical and physiological testing, investigators should develop techniques that can serve as biological indicators in the field as well as predictors in the laboratory to estimate the "health" of a particular aquatic resource. However, biochemical and physiological changes must be viewed in light of the degree and duration of change to determine whether the organism can adapt or whether the changes lead to irreversible homeostatic disturbances and finally to the death or debilitation of the organism.

BEHAVIOR

Any alteration in the ability of an organism to perceive and respond to its environment will affect its survival and may increase ecological mortality. Reports on behavioral changes induced by toxicosis cover an array of behaviors, and diverse techniques have been used to study these. The extent to which these methods can be applied in toxicological investigations depends on the economy of the procedure as well as on the accuracy with which behavioral changes can be quantified. Two contaminants, or even two concentrations of the same contaminant may affect different behavioral responses, and behavioral alterations caused by a substance may vary among species. Thus, toxicological studies should rely on multiple behavioral responses. The following behavioral responses are being evaluated as routine screening tests for the effects of various contaminants.

1. Avoidance - Aquatic organisms avoid certain comtaminants and are attracted by others. When a contaminant is introduced through either arm of a Y-maze, avoidance reactions have been shown to occur in mosquitofish (*Gambusia affinis*) to insecticides (Kynard 1974), in rainbow trout (*Salmo gairdneria*) to herbicides (Folmar 1976), in shrimp and mosquitofish to PCB's (Hansen *et al.* 1974) and in Atlantic salmon (*Salmo parr*) to heavy metals (Sprague 1964).

2. Predator-prey relationships - Various contaminants also disrupt predator-prey relationships by changing locomoter responses such as swimming or activity levels, or by disorienting the organism or by impairing its ability to perceive a predator or prey. Several studies have shown that the certain contaminants may increase the prey organism's vulnerability to predation (Goodyear 1972; Kania and O'Hara 1974; Tagatz 1976; Farr 1977; and Sullivan *et al.* 1978).

3. Feeding and swimming activities - The survival of recently hatched fry or invertebrate larvae depends in part on the time at which specific behavioral patterns develop. Delayed or inhibited behaviors such as feeding or swimming have been shown to occur as a result of contamination (Dill 1974).

Specific behavioral effects caused by contaminants are being correlated with other biological characteristics such as pathology, biochemical aberrations, or reproduction, as well as with the survival of aquatic organisms in natural systems. Also, the mechanism through which behavior has become altered in aquatic organisms exposed to pollutants is being examined.

ECOSYSTEMS

Field Studies

One of the least explored areas of either ecology or environmental toxicology is the ability of ecosystems to withstand contaminant stress. The use of pesticides in environmental management and the deposition of industrial contaminants in natural aquatic ecosystems has created a need for studies on the effects of these materials on biological communities. Laboratory studies can provide data on the effects of particular pesticides or contaminants on many species of organisms under various environmental conditions. However, such information may be of limited value at times in predicting the effects of pesticides and other contaminants on changes in biological communities where many species interact. Contaminants may modify these species interactions by affecting non-target organisms or be ecologically restructuring the biological community. These cause and effect ecological interactions in natural aquatic communities can be estimated by measuring certain characteristics such as primary productivity, standing crop, species diversity, community respiration, nutrient cycling, etc. in controlled lentic environments. Although chemical damage to a variety of ecosystems is at least partially documented, and, in fact, has constituted a major public and scientific concern in recent years, the facility with which ecosystems may resist or recover from the action of toxic compounds has received remarkably little attention.

The presence of a contaminant in an ecosystem, however, does not in itself imply toxicity. The contaminant must first be biologically available (Pavlou *et al*. 1977). Toxicity is the characteristic of an individual organism's response to a chemical at a particular concentration or dosage for a specific period of time. The effect of a contaminant on a community or ecosystem will depend, therefore, upon the summation of all individual responses within affected populations. Even though toxicity is generally most evident at the organismic and population level, community and ecosystem responses to organic contaminants can hypothetically be assessed directly or indirectly. The indirect approach is more probably within the present knowledge base of ecology and toxicology and involves the determination and monitoring of critical ecosystem processes. This approach is analogous to the medical one where the disease or malfunction is ascertained by a set of symptoms. Symptoms are functional evidences of disease, and the observance and measurement of symptoms may be far removed from the actual affected organ(s) or system.

Evaluation of the impact of contaminants on aquatic organisms has been limited mainly to laboratory studies. Much of the laboratory research lacks field verification and the true impact of contaminants on aquatic organisms in the wild is poorly understood. The classical field approach involves laborious age, growth, and population dynamics studies of fish and extensive surveys of other flora and fauna (species diversity) that would probably be applicable to that time and place only. Also, field studies are somewhat limited to effects evaluation after contamination has occurred and can provide only limited predictability (Brungs and Mount 1978).

One of the main objectives of recent research has been to establish the necessary measurements essential to predicting pesticide and other contaminant effects on lentic ecosystems (Boyle 1979a,b). In experimental ponds exposed to herbicides (2,4-D DMA, dichlobenil, and fenac), one to seven characteristics were sufficient to explain 80-90 percent of the differences observed. The seven characteristics found to be most important were pH, alkalinity, turbidity, total dissolved nitrogen, total phosphorus, chlorophyll a, and zooplankton density.

Biochemical Characteristics of Ecosystem Stress

The onset of environmental change in aquatic systems due to stress imposed by man is often difficult to discern. Even after severe ecological damage has occurred, substantiation requires the collection and evaluation of voluminous amounts of data. Train (1972) has pointed to the need for usable indicators of environmental quality. Indicators of ecological stress would be especially useful if they could be applied at the beginning of ecological disasters, rather than proof that extensive ecosystem change has already occurred. Although there is no well developed literature on this subject, several studies indicate the possibility of using chemical and biochemical characteristicss as indicators of ecological stress. Woodwell (1972) cites three qualities of stressed ecosystems, (1) simplification of structure; (2) shifts in the ratio of production to respiration; and (3) loss of inorganic nutrients. Some marine studies have linked specific biochemical characteristics with ecological change (Jefferies 1972; Jefferies and Alzara 1970), but similar references are not apparent in the literature in freshwater. The changes in some chemical variables, such as concentration and location of inorganic nutrients, total organic matter and biochemical diversity, seem to offer an opportunity to construct a set of symptoms for early detection of ecological contamination. Interpretation of the significance of field-measured changes, however, requires realistic physiological and biochemical studies under experimental conditions. It also requires development and adaptation of chemical methods for measurement of contaminants in biota, sediment, and water.

RESIDUE DYNAMICS AND BIOCONCENTRATION

Factors that control the flow of contaminants through an ecosystem have been classified into four major areas: (1) Physical transport and spatial distribution; (2) Interfacial processes; (3) All noninterfacial chemical transformations exogenous to the biota; and (4) Biotransformations (Pavlou *et al*. 1977).

The physical transport and spatial dispersion are ecosystem specific and depend on the circulation and flow dynamics associated with the dispersive medium. These aspects have been discussed extensively by Gillet et al. 1974).

Interfacial processes can be broken down into two categories: (1) Interfacial interactions not involving changes of the contaminant, but which result in the exchange of the compound with the dispersive medium (soil, water and air), and (2) all chemical reactions, abiotic or biotic, that alter the chemical structure of the compound. Interfacial interactions not involving changes of the comtaminant include volatilization, dissolution and sorption (adsorption and absorption), molecular associations such as chelation, hydrogen bonding, ionic interactions, etc. These physico-chemical interactions are important because contaminants may not only be immobilized, but that can also mediate mobilization and transport as reported by Ogner and Schnitzer (1970). Also, the interactions are amenable to classical physico-chemical treatment and interpretations. In addition, chemical structure is a crucial aspect, not only as a flow-factor, but also in toxicity (Addison and Cote 1973; Cohen et al. 1974; Kapoor et al. 1973; Kopperman et al. 1974; Sugawara 1974; Vilceanu et al. 1972; Wildish 1974).

Studies on abiotic noninterfacial transformation reactions (photodegradation, hydrolysis, etc.) have been conducted for only a few organic compounds (Crosby and Leitis 1973; Crosby and Moilanen 1973; Crosby and Moilanen 1974; McGuire et al. 1970; Pope et al. 1970; Pope and Zabik 1970; Ruzo et al. 1972; Zabik et al. 1971). Consequently an assessment of their importance to ecosystem transport and availability is virtually impossible. However, the results obtained from certain toxicological investigations involving pesticides suggest that biotransformations may activate or deactivate the parent compound to more or less toxic metabolities (O'Brien 1967; O'Brien and Yamamoto 1970). Since the biological availability of organic chemicals is of critical importance to evaluating toxicity, and thereby potential ecosystem malfunction, the development of useful transformations and interfacial exchange features has been undertaken.

The degree of bioaccumulation as a function of the available concentrations in the medium can be predicted. Recent studies by Neeley et al. (1974) have shown that the octanol/water partition coefficients for organic chemicals are linearly correlated with bioaccumulation in fish. Correlating the octanol/water quantities and environmental concentrations for a series of chemicals may prove useful in providing a rapid screening technique for predicting environmental concentrations. In addition, computerized treatment of residue data from aquatic organisms continuously exposed to contaminants is actively being developed. The uptake phase is usually 28-56 days and the elimination phase is 28 days (Figure 1). Accelerated bioconcentration tests of only 4 days have been used with some chemicals to predict bioconcentration under longer exposures (Branson et al. 1975).

ENVIRONMENTAL HAZARD EVALUATION

The Toxic Substances Control Act of 1976 clearly indicates that an "unreasonable risk" of injury to health or the environment caused by manufacture, distribution, use, or disposal is needed to establish a chemical as

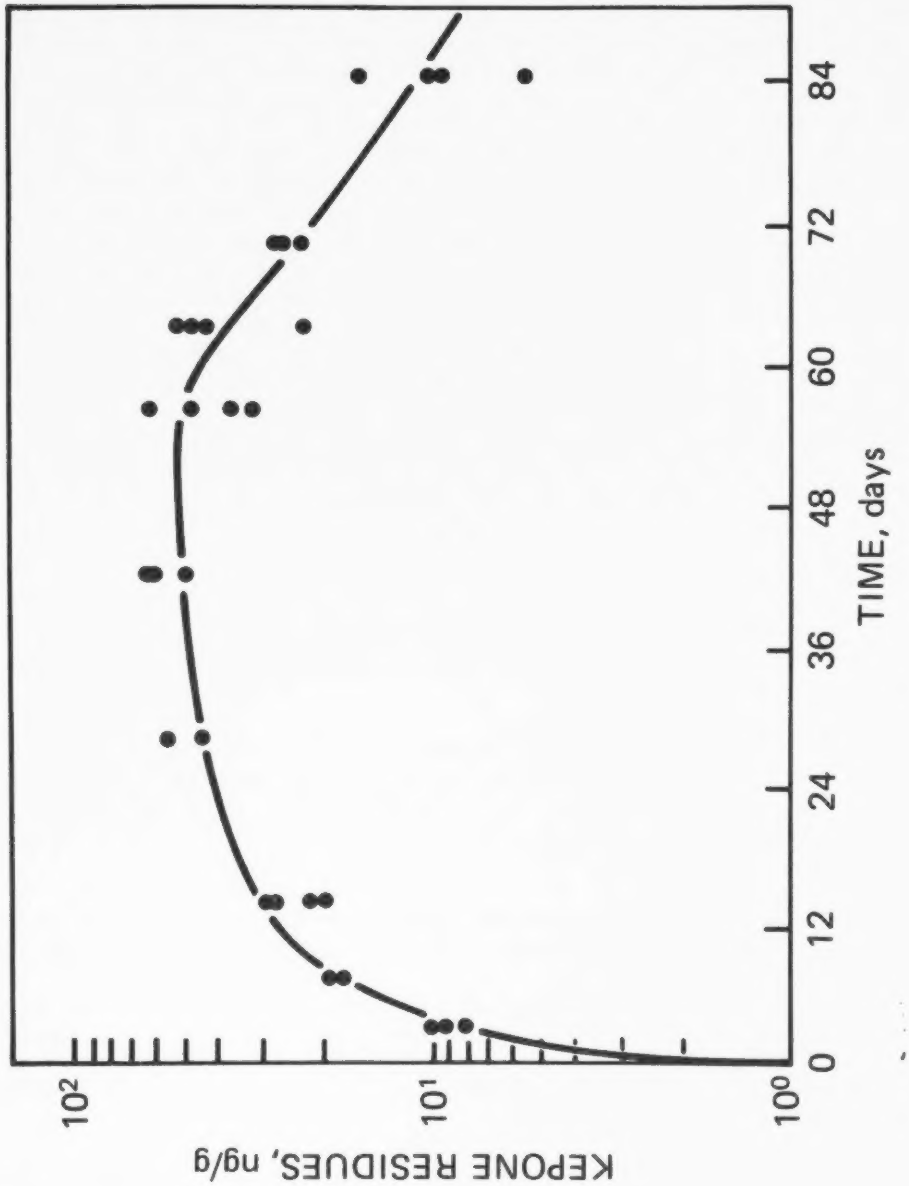

Figure 1. Computerized treatment of residue data from fathead minnows exposed to 3.7 ng/l of Kepone. Fish were continuously exposed for 56 days and placed in uncontaminated water for 28 days. Parameter estimates:

Time to reach 90% of steady state	43 days
Bioconcentration factor	15,053
Time for 50% elimination	13 days

hazardous. Hazard evaluation is a probability assessment that adverse ecological effects will result from environmental releases of a given contaminant. It involves a sequential and integrated approach to predict the safety or hazard of the contaminant, and includes information on (1) chemical production, use, and disposal patterns; (2) acute and chronic toxicity; (3) residue dynamics and bioconcentration; (4) environmental fate and monitoring; and (5) field studies (Figure 2). A hazard evaluation is not a one-time estimate, and additional evaluations must be made as the data base expands. Useful assessment schemes have recently been proposed by Kimerle _et al._ (1978), Duthie (1977), Stern and Walker (1978), and the American Institute of Biological Sciences (1978). However, no scheme or procedure can eliminate the need for sound scientific judgement. The evaluation, in its essence, is a scientific judgement of the potential for environmental effects (toxicity tests) with measured (or estimated) environmental concentrations. The degree of confidence in the evaluation is greatest with a reliable estimate of environmental concentrations and with effects data which includes studies on representative species under conditions simulating those of natural aquatic environments.

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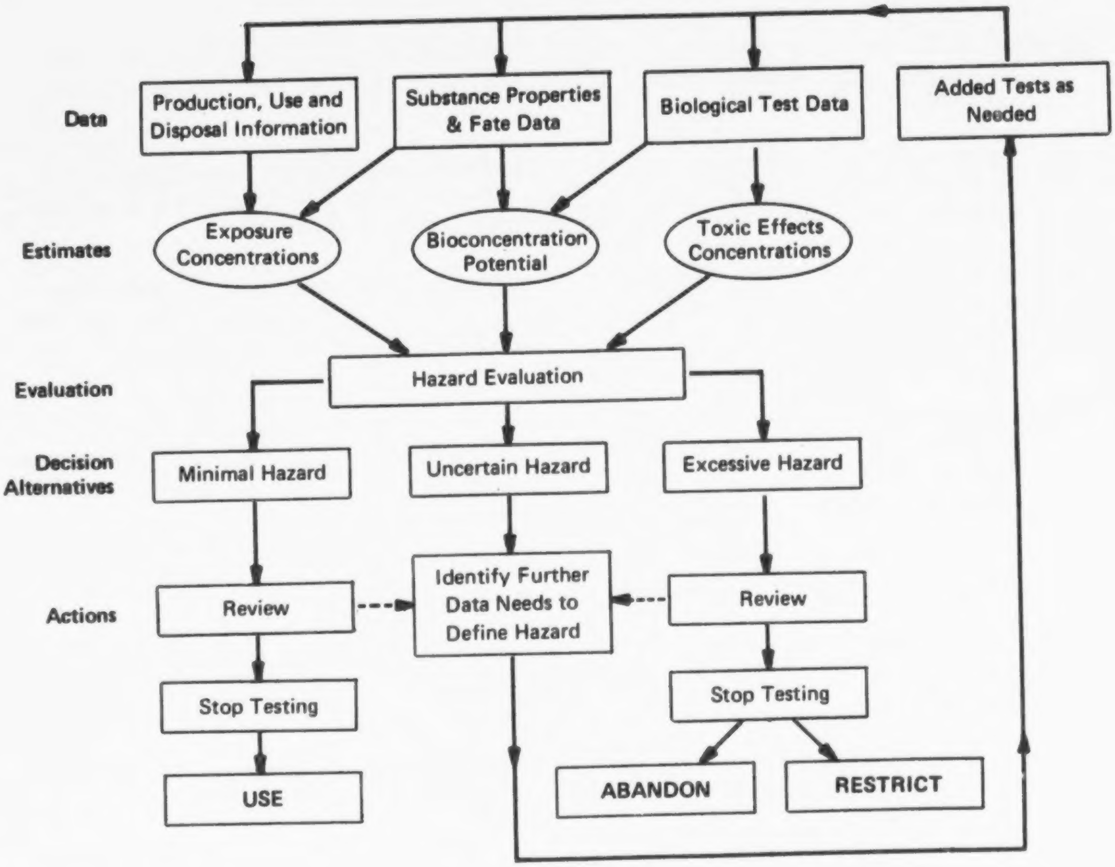
Data
Production, Use and Disposal Information
Substance Properties & Fate Data
Biological Test Data
Added Tests as Needed
Estimates
Exposure Concentrations
Bioconcentration Potential
Toxic Effects Concentrations
Evaluation
Hazard Evaluation
Decision Alternatives
Minimal Hazard
Uncertain Hazard
Excessive Hazard
Actions
Review
Identify Further Data Needs to Define Hazard
Review
Stop Testing
Stop Testing
USE
ABANDON
RESTRICT

Figure 2. Schematic diagram of the environmental hazard evaluation process (modified from American Society of Testing and Materials Hazard Evaluation Task Group, J.R. Duthie, Chairman).

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SECTION 5

COMPARISON OF PRINCIPLES OF DEVELOPMENT AND USE OF WATER QUALITY STANDARDS IN THE USSR AND USA

L.A. Lesnikov[1]

Practically all nations, which have experienced the negative influence of pollutants from industry and agriculture on bodies of water, have arrived at the need to establish certain standards for these substances which are considered safe for the use of bodies of water (McKee and Wolf, 1963).

However, in developing biological well-founded standards, a primary difficulty arises: the development of sufficiently well-founded standards is quite cumbersome, while the number of pollutants which may enter bodies of water is quite great. As we learned on a visit to the USA, the "bank of substances" at one laboratory in Cincinnati includes some 25,000 substances. In our country, about 600 sanitary-hygienic maximum permissible concentrations (MPC) have been developed for harmful substances, as well as 210 fishing industry MPC's. In the USA, judging from the literature which we have examined, reports have been published on the degree of harm of a similar quantity of substances, though as yet this information has primarily been obtained from short-term experiments. Large numbers of substances have been studied in both the USSR and the USA. Summing up all the information which we have available at present, we know of the effect of only about 1,000 substances.

The following system is used in the USSR. MPC's are the same for all bodies of water in the country, but there are two systems of MPC's: sanitary-hygienic, approved by the USSR Public Health Ministry, and fishing standards, approved by the Fishing Industry Ministry, USSR. These standards must be maintained by enterprises, beginning at a "measurement line" and beyond it. For the sanitary-hygienic MPC's, the "measurement line" is 1 km upstream from the nearest point of water use in the case of rivers, or 1 km distant from the nearest point of water use for reservoirs and lakes. For the fishing standards, the "measurement line" is established no more than 0.5 km from the source of pollution.

For each specific enterprise, "discharge norms" or, as they have come to be called in recent years, "maximum permissible discharges" (MPD) are established, i.e., the calculated quantity of any polluting substances, both as to concentration and as to total volume, which can be discharged without disrupting the MPC at the measurement line.

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Sanitary-hygienic MPC's are not the subject of the present report, but we note that, as they are developed, both short-term and long-term effects of substances on the sanitary condition of bodies of water are considered (the oxygen regime, content of substances capable of decomposition, capacity of the water for stagnation and self-purification, number of microorganisms, etc.), on the organoleptic properties of water, on the health of the local population (toxicity, pathogenic organisms, etc.) (Cherkinskiy, 1971). In the past decade, the stability of the pollutants and their cumulative properties have also come to be considered.

The fishing MPC's require study of: the stability of the pollutant, its influence on the sanitary status of the reservoir (transparency, color of water, pH, oxygen regime, BOD, etc.); the organisms of phytoplankton, aquatic microorganisms, zooplankton, zoobenthos, spawn, larvae and mature fish; cumulation of the substance by fish; and the influence on the quality of fish flesh. Approximate times of experiments were presented by us in our previous report (Lesnikov, 1976).

In analyzing the materials which we have received from our American colleagues, we at first thought to compare all available materials, but then decided to concentrate our attention on research on fresh-water organisms, since water toxicologic studies on marine organisms have not yet been sufficiently developed in the USSR (Patin, 1977) to speak of the relative toxicity resistance of species. Therefore, the results of USA studies on marine organisms shall be included only as is convenient.

In the USA, the degree of danger of a substance for fish and other aquatic organisms, as determined experimentally, is summed up in the integral indicator "water quality criterion". According to McKee and Wolf (1963), this indicator is considered in the establishment of "water quality standards" for specific areas of bodies of water. The specifics of use of the body of water and relative toxicity resistance of the species which inhabit it are considered.

In order for one nation to use data obtained by another nation, it is necessary to gain some idea concerning the relative toxicity resistance of test organisms. Naturally, representatives of local aquatic fauna are used both in the USSR and in the USA.

In our country it is the usual practice to divide organisms into four groups in terms of their relative toxicity resistance (oligotoxobes, betamesotoxobes, alphamesotoxobes and polytoxobes) (Lesnikov, 1976). We shall attempt to classify the test organisms used for toxicologic research in both the USSR and USA from this standpoint. It must be considered that this classification is somewhat arbitrary, since the toxicity resistance of organisms varies for various toxic substances. It is more correct to speak only of trends. The relationship of sensitivity also varies as a function of the duration of exposure. We shall present here data obtained by the ichthyopathologist of our laboratory, O.N. Krylov (1973) on the influence of chlorophos (Dipterex) on fish (see Table 1).

TABLE 1. RELATIONSHIP OF LT_{50} (mg/liter) OF CHLOROPHOS FOR CURRENT YEAR'S BROOD OF FISH AS A FUNCTION OF TIME OF EXPOSURE

Exposure	*Coregonus peled*	*Salmo irideus*	*Gasterosteus aculeatus*	*Cyprinus carpio*
96 hours	0.24	0.78	6.0	282.0
25 days	0.031	0.062	0.25	2.0

With an exposure of 96 hours, *Coregonus peled* was 1200 times more sensitive to chlorophos than *Cyprinus carpio*, while with an exposure of 25 days, it was only 64 times more sensitive. As a rule, the longer the exposure, the less the difference is between sensitivities of species.

Our ideas concerning the relative sensitivity of test organisms to toxic substances are presented in Table 2. The relative sensitivity of the test organisms used in the USSR is estimated on the basis of studies of the GosNIORKh Water Toxicology Laboratory (Lesnikov, 1976, 1973; Krylov, 1973; Alekseyev and Lesnikov, 1977; Stroganova, 1971), while the relative sensitivity of test organisms used in the USA is based on the works of McKee and Wolf, 1963, Mayer *et al*., 1975; Meerle and Mayer, 1975; Sanders, 1977; Sanders *et al*., 1973; Mayer *et al*., 1976, 1977; Carlson, 1972; Hermanutz *et al*., 1973; Macek *et al*., 1976; Sauter *et al*., 1976; Snarski *et al*., 1976; Allison and Hermanutz, 1977; Pickering *et al*., 1977; Christensen *et al*., 1977; Eaton *et al*., 1978; McKim, 1977; McKim *et al*., 1976; Benoit *et al*., 1976; Carwell *et al*., 1977; Spehar, 1976, Spehar *et al*., 1978; Hermanutz, 1977; McKimm *et al*., 1978; Lloyd, 1976; Lloyd *et al*., 1976. Of course, this table must be considered a first approach to the problem. We can see from the data presented that some organisms, e.g., *Salmo irideus*, *Cyprinus carpio* and *Daphnia magna*, are used in both countries, while the others are similar in their sensitivity. At the present time, neither country uses the most toxicoresistant species. Consequently, the data compared using today's test organisms are comparable.

The experimental differences are small in most cases, significant in a few cases.

EXPERIMENTS ON FISH

In the USSR, experiments are performed on eggs, larvae, current year's brood and second year fish, less frequently on older fish. The usual duration of acute experiments is not over 15 days. As in the USA, the LC_{50} is determined for 96 and 120 hours, and the curve of median lethal time as a function of substance concentration is studied. Subacute experiments, allowing the boundary of chronic lethal effect to be determined and sublethal effects to be revealed, last up to 3 months (90 days). Chronic experiments,

TABLE 2. RELATIVE TOXICORESISTANCE OF FRESH-WATER TEST ORGANISMS USED IN TOXICOLOGIC EXPERIMENTS IN THE USSR AND USA

	USSR			USA		
	Toxicoresistance			Toxicoresistance		
	Low	Medium	High	Low	Medium	High
Fish	Coregonus peled Salmo irideus S. salar S. trutta Acipenser ruthenus	Phoxinus phoxinus Esox lucius Stizostedion (Lucioperca) lucioperca Perca fluviatlis	Cyprinus carpio	Salvelinus fontinalis Salmo hairdneri Coregonus nasus Salmo trutta Onchorhynohus kishuth	Pimephales promelas Ictalurus punctatus Lepomis macrochirus Catostomus commersoni Jordanella floridae Esox Stizestedion v.vitreum	Cyprinus carpio Carassius auratus
Phytoplankton		Scenedesmus quadricauda			Scenedesmus quadricauda Selenastrum capricorneum	Chlamidomonas sp.
Zooplankton	Daphnia loqispina D. magna	Daphnia pulex	Cyclops strenuus Paramecium caudatum	Daphnia magna		

TABLE 2. (CONT.)

	USSR			USA		
	Toxicoresistance			Toxicoresistance		
	Low	Medium	High	Low	Medium	High
Zoobenthos		Gammarus pulex G.lacustris Asellus aquaticus Cloeon	Radix stagnalis R.auricularia Baetis sp.	Hydropsyche betteny Ephemerella sp. Procambarus clarkii Cambarus diogenes Ephomera similans	Asellus brevicaudatus A.militans Chironomus plumosus Gammarus pseudolimnaeus Hexaqenia bilineata Ischnura verticalis Hyallela octeca Pteronarcis dorsalis Physa inteqra Baetis vagans	

performed to answer questions similar to those answered by subacute experiments, last up to 6 months or more.

The influence of the substance on survival, growth in length and weight, development of eggs and larvae are all considered. The pathoanatomic and pathohistologic changes in the organs and tissues (liver, kidneys, gut, brain, sometimes spleen, gills, blood - hemoglobin, formed blood elements, sometimes blood protein) are also considered.

In the USA, experiments are also performed on eggs, larvae, current year's brood and mature fish. Furthermore, experiments have been undertaken modeling the spawning of fish, extending over three generations: sexually mature fish, the production of eggs and larvae which mature to the reproductive state themselves, observations on eggs and the larvae which they produce. In many cases, the experiments extend over 2-3 months and may be compared to the "subacute experiments" in the USSR, but in many cases the length of these experiments is greater than for chronic experiments in the USSR - up to 1-3 years. Most experiments, however, last 90-150 days, i.e., comparable in length to those conducted in the USSR.

The same indexes are considered as in the USSR: survival rate, growth in length and weight, development of eggs and larvae, but also the influence of the substance on spawning of the fish is determined. Similar studies should be organized in the USSR as well. Furthermore, in the USA a successful "proportional diluent" scheme has been developed (Brungs and Mount), which is quite convenient in the performance of chronic experiments. In the USSR, new solutions are regularly prepared and manually replaced. Development of a standard diluent is desirable for our country.

Of the histopathologic analyses, we found only one work in the USA (Couch, 1975) which included information on changes in the liver of fish.

Thus, the results of ichthyotoxicologic experiments in the USSR and USA are basically comparable.

EXPERIMENTS ON ALGAE

In the USSR, the most commonly used test organism of algae is _Scenedesmus quadricauda_, sometimes _Chlorella vulgaris_, with other species used only in special studies (Khobot'yev and Korol', 1971; Khobot'yev _et al_., 1971; Kohbot'yev and Kapkov, 1971; Mosiyenko, 1974a, 1974b; Pain and Tkachenko, 1974; Vislyanskaya and Vedyagina, 1974; Lisovskaya _et al_., 1968). Due to the difficulty involved in replacement of the medium (difficulty in separation of algae from the liquid), the substance being studied is introduced to the medium once, or a portion of the medium is replaced with fresh solution, with an additional quantity of the toxicant introduced. The usual duration of experiments is 25-30 days. Indexes recorded include: dynamics of population of algae, settling rate, influence on pH of medium, on liberation of oxygen, sometimes on absorption of radioactive carbon.

In the USA, toxicologic experiments are performed on Selenastrum capricorneum (Bartlett et al., 1974; Ferris et al., 1974), Chlamidomonas sp. (de la Cruz and Nagvi, 1973); we found more detailed experiments on marine algae (Walsh, 1972; Walsh et al., 1977), judging from which the indexes considered are the same as in experiments performed in the USSR, but the duration of exposure is shorter--7-10 days. Considering the differences in experimental duration, the results of the experiments are quite comparable.

EXPERIMENTS ON ZOOPLANKTON ORGANISMS

The main test organism in both countries is Daphnia magna. In the USSR, experiments are performed in two variants:

1. According to the system of Professor N.S. Stroganov, on three or more successive generations of crustacea, the experiments with each generation lasting 20-21 days (Stroganov, 1971; Stroganov and Kolosova, 1971; Lesnikov, 1973). The indexes observed are: survival rate, growth, intensity of reproduction and quality of progeny. In addition to these indexes, the nature of processes of oogenesis and embryogenesis, body color, accumulation of droplets and fat and their color, degree of filling of the gut and color of its contents and others are sometimes considered (Lesnikov, 1971).

2. According to the system of Lesnikov, using populations of daphnia. This differs from the previous method in that the young which are born are counted but are not removed from the experimental vessels (the most convenient capacity of which is 1 liter). The duration of the experiments is until the maximum biomass is obtained in the control and in the vessel containing the substance being tested at the lowest concentration, usually 20-30 days; sometimes experiments are continued until the second or third peak of biomass (usually 50-60 and 70-120 days). The indexes considered are the same as in experiments on series of generations and, furthermore, consideration of biomass of the daphnia and the change of parthenogenetic reproduction to bisexual reproduction. Incidentally, it has been determined that the influence of sublethal concentrations of a number of substances is manifested in that the daphnia do not go over to the bisexual method of reproduction at the usual time or defective latent eggs are formed which later burst.

In the USA, experiments on Daphnia magna are performed according to a plan quite similar to that of N.S. Stroganov (Sanders, 1977; Sanders et al., 1973; Carwell et al., 1977). The time of experiments on one generation is 21-28 days; in experiments on series of generations, the times are approximately the same for each generation (Macek et al., 1976).

The results of the experiments are fully comparable.

EXPERIMENTS ON BENTHIC INVERTEBRATES

In experiments with this group of organisms, a great variety of test organisms is used in both countries, the USSR and the USA.

In the USSR, various species of fresh-water gammaridae are used (Gammarus pulex, G. lacustris, Pontogammarus robustoides, etc., Asollus aquaticus), of the insects - Chironomidae, most frequently Chironomus dorsalis, for which a method has been developed of year-round cultivation under laboratory conditions (Konstantinov, 1958). Remaining species of the mollusks, ephemeroptera and odonata are less frequently used.

Experiments with gammaridae are performed over a period of approximately a month, considering survival, intensity of cannibalism, growth and multiplication of the crustacea and their feeding rates.

Experiments with Chironomidae extend from emergency of the larvae to flight of the imagoes. Survival rate of larvae, pupae and imagoes are noted (Bugayeva, Puzikova, 1974).

In experiments on other invertebrates, survival rate and growth are usually noted, sometimes breeding rate as well.

In the USA, similar groups of benthic organisms are used. One specific factor is the use of several ephemeroptera (Baetis vagans, Ephemera similans, Hexagema lineata), species which are rather sensitive to toxins. However, differences are observed. Our experiments with Baetis sp. (species not precisely defined) have shown that this form was tolerant to methylnitrophos, sevin and cobalt chloride. The American species (Baetis vagans), judging from the results of experiments, has at least moderate sensitivity (experiments of Lloyd et al., 1976). In the USA, experiments are performed on the larvae of Plecoptera (Pteronarcis californica, Acroneura pacifica) (Sanders and Cope, 1968). Judging from the figures they present, these species are moderately, possible highly sensitive to toxins. Of the Chironomidae, Tanytarsus is used in the USA (in the laboratory at Duluth). According to GosNIORKh, Tanytarsus is somewhat more sensitive, at least to chlorophos, than is Chironomus.

Thus, there are no basic differences in the methods used in experiments on benthic organisms in the USSR and USA, and there are no great differences in the relative sensitivities of the test organisms used.

The greatest differences are observed in methods of estimation of the influence of pollutants on microorganisms and on the hydrochemical mode.

INFLUENCE OF POLLUTANTS ON AQUATIC MICROORGANISMS

In the USSR, experiments are performed in aquaria, to which fixed concentrations of the substances studies are added (once), then the dynamics of the population of microorganisms are observed (total count on membrane filters, population of saprophytes growing on MPA) as well as the numbers of specific groups of microorganisms which may be encountered, judging from the nature of the substances studied, e.g., cellulosolytic bacteria for the sewage of cellulose-paper plants, petroleum oxidizing bacteria when studying petroleum-containing waste water or specific petroleum products, etc. Experimental durations are 21-30 days (Mosevich, 1973). These experiments have been included in a large system of studies, mainly performed in laboratories of the GosNIORKh systems, though other water toxicology laboratories do not always include them, since they do duplicate hydrochemical experiments to some extent.

It has been found that when water from natural bodies of water is placed in aquaria, during the first four days a significant increase in the population of microorganisms is observed, after which the number of organisms varies within limits characteristic for the conditions in question. During this time, the water from the natural body of water becomes aquarium water.

The effects of pollutants may result in an increase in the total population of microorganisms, or of certain specific groups, or may suppress bacteria processes.

In the USA, based on the articles available to us, only one work (Duthrie *et al*., 1974) is similar in methodology to works in the USSR: experiments to determine the effect of diuron on microbial processes were performed in experimental tanks. In the Laboratory for Study of Environmental Pollutants at Gulf Breeze, Florida, a basically different system of studies in "microcosms" (glass pipes containing water and soil) is used (Bourquin, 1977; Bourquin *et al*., 1977). The duration of these experiments is also 20-30 days, but the results are basically different. Each experimental system has its advantages and disadvantages; therefore, the comparability of results of these studies requires further checking.

HYDROCHEMICAL EXPERIMENTS

Studies are performed according to two main systems.

1. Estimate of intensity and nature of breakdown of pollutants.
2. Influence of pollutants on hydrochemical regime of bodies of water, particularly processes of self-purification from substances other than the pollutant itself.

Studies of the breakdown or the fate of the pollutant in the water system have been undertaken in both the USSR and USA to varying degrees in almost all experiments. In laboratories of the GosNIORKh system, chemical determination of the eventual fate of the pollutant are always accompanied by biological toxicologic tests, usually using *Daphnia magna*. Frequently, the products of decomposition of the substance are more toxic than the substance itself. For example, experiments in our laboratory have determined that in solutions of chlorophos (Dipterex) in natural water, during the first 2-5 days, the mean survival time of Daphnia decreases to half; this elevated toxicity is retained for 1.5 months in open vessels and up to 2 months or more in closed vessels. This phenomenon can be attributed to DDVP (dimethyldichlorovinylphosphate), a product formed upon decomposition of chlorophos (dimethyloxytrichloroethylphosphonate). An increase has been found in toxicity during the first week in solutions of orthoxylene, though the mechanisms of the process itself is not clear.

Of works of this type performed in the USA, we would like to note an exceptionally interesting study by Mancy and Allen (1977), on the influence of environmental factors on the toxicity of heavy metal ions.

A second trend is estimation of the influence of a pollutant on the hydrochemical processes in a body of water. This type of experiment is an obligatory component of all water toxicology studies in the USSR. We found no analogous studies in the USA. In these experiments, water is taken from a natural reservoir and placed in an aquarium for study. In our laboratory, water is taken from a reservoir with hard water (e.g., the Strelka River) and another body of water with soft water (e.g., Lake Ladoga). A series of concentrations of the pollutant, usually 6-7 gradations, is created, with pure water serving as the control. Analysis of pH, dissolved oxygen, BOD_5, BOD_{20}, permanganate and bichromate oxidizability, forms of nitrogen (ammonia, nitrates, nitrites) are regularly analyzed, and changes in the concentration of the pollutant are observed. Many substances cause a decrease in the content of dissolved oxygen and an increase in BOD, increasing the saprobic nature of the medium. Toxic substances may significantly suppress, either temporarily or throughout the experiment (usually 25-30 days) processes of self-purification. Most frequently, processes of oxidation nitrogen are first suppressed, i.e., processes of formation of nitrites from ammonia compounds and oxidation of nitrites to nitrates. In many cases, an increase is found in the content of nitrites which cannot be explained by oxidation of ammonia compounds and can be attributed only to denitrification processes.

Summing up all that we have said, we note that, with the exception of a small number of tests used in one country and not in the other, the studies in the two countries, the USSR and the USA, generally follow the same goals, and at the present time are performed according to basically similar methods, which is determined as we compare works performed in the two countries. The most difficult question is that of the maximum permissible standardization of a minimum program of these investigations.

It is hardly necessary to change the forms of application of the standards developed in one or the other of the countries--they are determined by the specifics of our individual national systems. We can simply state that the MPC system used in the USSR is equivalent in the nature of its scientific foundation to the concept of the "water quality criterion" used in the USA, while the water quality "standards" used in the USA are more or less equivalent to the "discharge norms" or "maximum permissible discharges" (MPD) used here.

The system of distribution of test organisms in terms of their relative sensitivity to pollutants represents some difficulty, since the relationship of sensitivity of species to various substances differs somewhat. At one time, Professor N.S. Stroganov suggested that the relative sensitivity of test organisms be estimated on the basis of the ratio to that of Daphnia magna; this can be done in works in both countries, since this species is used in experiments in both the USSR and the USA. In any case, the system which we have proposed (Table 2) should be looked upon as simply a first approach to the problem and should be refined as data are accumulated.

Thus, there is a firm basis for successful cooperation of both nations in the development of specific means for protection of bodies of water from pollution.

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SECTION 6

CHLORINATED HYDROCARBONS AS A LIMITING FACTOR IN THE REPRODUCTION OF LAKE TROUT IN LAKE MICHIGAN[1]

Wayne A. Willford[2]

THE FISHERY

From about 1890 until 1945, the lake trout (Salvelinus namaycush) was the most valuable and sought-after commercial species in Lake Michigan. The annual commercial catch averaged 8.2 million pounds (3,700 metric tons [t]) from 1890 to 1911, 7.0 million pounds (3,200 t) from 1912 to 1926, and 5.3 million pounds (2,400 t) from 1927 to 1939. The catch increased slightly to an annual average of 6.6 million pounds (3,000 t) during 1940 to 1944, but then began to decline precipitously in 1945 and had fallen to only 342,000 pounds (155 t) by 1949 (Figure 1). In 1954, the catch was a mere 34 pounds (15 kg), and by 1956 the species was probably extinct in Lake Michigan (Wells and McLain 1973).

The gradual decline in the commercial harvest of lake trout from 1893 to 1938 is believed to have resulted from excessive exploitation (Van Oosten 1949; Wells and McLain 1973). Although the commercial harvest of lake trout continued into the early 1950's, the apparent extinction of the species in about 1956 is believed to have been caused directly by the predatory sea lamprey (Petromyzon marinus), an exotic species that became firmly established in Lake Michigan in the decade following its first reported presence there in 1936 (Wells and McLain 1973).

Early attempts to control the sea lamprey consisted of installing electrical and mechanical barriers, which blocked the spawning runs of adults. Between 1953 and 1958, barriers were constructed across 65 tributaries flowing into Lake Michigan. At about the same time (in the late 1950's) a successful lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), was discovered and developed by scientists at the Hammond Bay Biological Station of the U.S. Fish and Wildlife Service (USFWS). This compound was soon being used to kill larval sea lampreys (ammocoetes) in tributary streams before they could metamorphose and migrate downstream into the lake. Most barrier operations were discontinued in 1960 in favor of TFM treatments, thus set-

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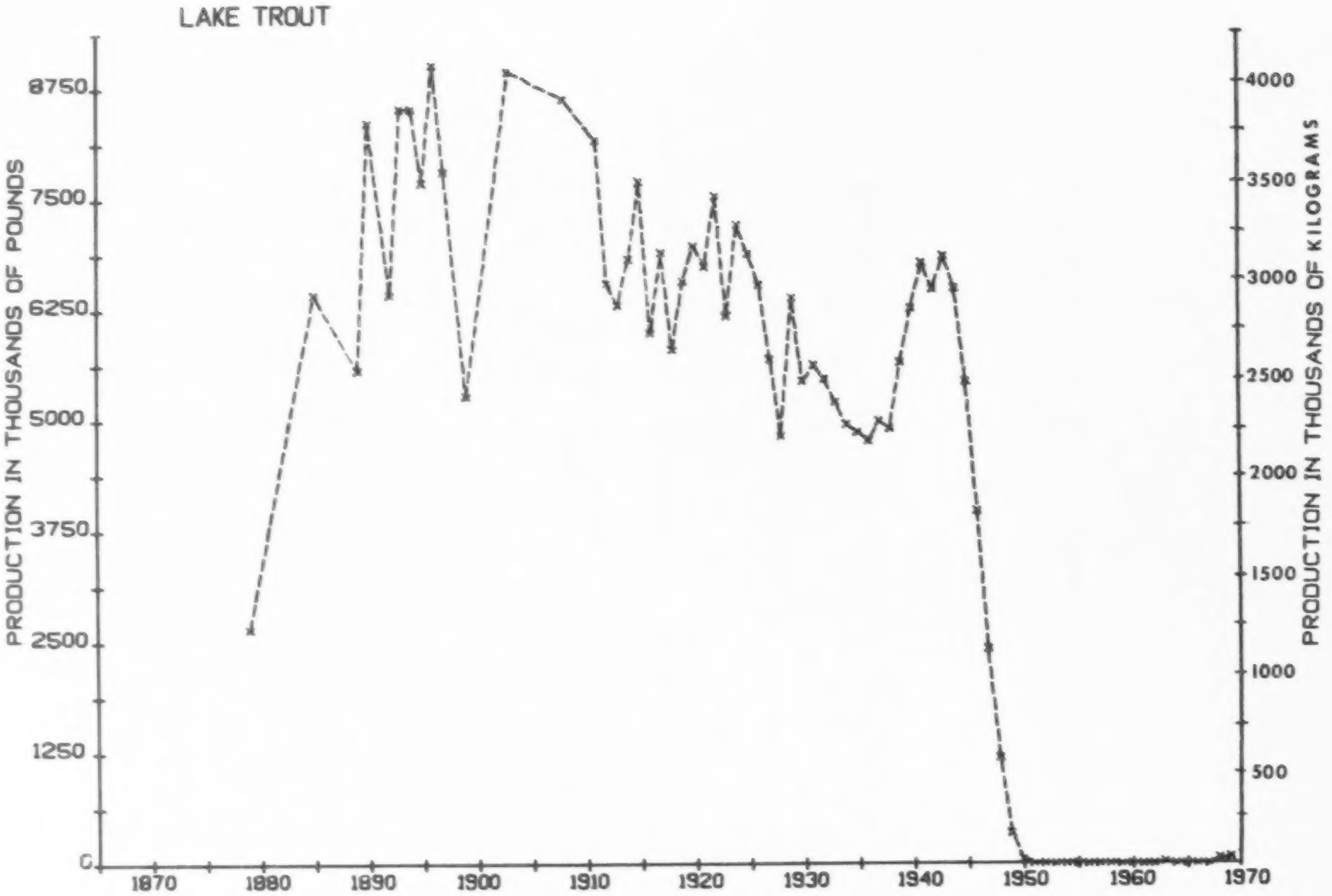

Figure 1. Commercial production of lake trout in Lake Michigan (Wells and McLain 1973).

ting the stage for the highly successful sea lamprey control program which followed. This program and the ongoing lake trout restocking program, which began in 1965 in Lake Michigan when about 1.3 million yearling lake trout were planted, have been coordinated by the Great Lakes Fishery Commission. In 1965-78, an average of over 2 million fin-clipped lake trout were planted in the lake each year (data provided by the Great Lakes Fishery Commission) as part of an effort to restore lake trout stocks to self-sustainability.

By the early 1970's, the lake trout were once again considered abundant in Lake Michigan and spawning activity was widespread each fall (Wells and McLain 1973; Great Lakes Fishery Laboratory 1974). Nevertheless, no naturally produced fingerling or older lake trout (recognizable by their lack of clipped fins) have been found in the lake during routine assessment sampling (Great Lakes Fishery Laboratory 1978). Therefore, little progress has been made toward the goal of rehabilitating self-sustaining stocks of lake trout, even though the lake contains a large population of mature fish that should be capable of reproducing naturally.

REHABILITATION PROBLEMS

Following the reports of widespread spawning of lake trout in the early 1970's, concern deepened about the apparent failure of the fish to produce surviving progeny. Numerous theories have been proposed to account for this reproductive failure, including the following:

1. Contamination of the water and fish by toxic substances such as pesticides and industrial chemicals;

2. Deterioration in bottom conditions on spawning reefs as a result of eutrophication and possibly increased sedimentation;

3. "Abnormal homing" of planted trout as spawning adults to their planting sites--generally shallow, inshore areas that offer little suitable spawning substrate and are vulnerable to sedimentation or scouring action by waves and ice;

4. Predation on, or feeding competition with, young lake trout by the now abundant, introduced species, rainbow smelt (Osmerus mordax) and alewife (Alosa pseudoharengus);

5. Artificial selection, extensive inbreeding, or physiological and behavioral conditioning of hatchery fish which somehow resulted in their inability to spawn successfully or to produce young that are capable of surviving in the wild; and

6. Insufficient "critical mass" or numbers of mature lake trout in the lake to permit the realistic expectation of successful reproduction in the early 1970's.

Various studies addressing these theories were soon initiated by the Michigan Department of Natural Resources (MDNR) and the USFWS Great Lakes

Fishery Laboratory (Rybecki and Keller 1978). Of greatest concern initially was the problem of toxic substances. The fish were known to contain substantial residues of DDT and its metabolites and of PCBs (Reinert 1970; Stalling and Mayer 1972). Concentrations of each of these contaminants exceeded 10 μg/g in adult lake trout (Willford 1975) and 4 μg/g in their eggs (Reinert and Bergman 1974). Published reports on the effects of DDTs (DDT, DDD, and DDE) and PCBs indicated that the concentrations of these contaminants in lake trout and their eggs were sufficient to interfere with reproduction. For example, Burdick et al. (1964) reported that concentrations of DDTs in excess of 2.9 μg/g in the eggs of lake trout resulted in increased mortality of fry. This effect was later confirmed by Macek (1968) who studied brook trout (Salvelinus fontinalis) fed DDT. Unusually high mortality of fry of coho salmon (Oncorhynchus kisutch) hatched from eggs of Lake Michigan fish, and possible correlation of that mortality with elevated levels of DDTs and other chlorinated hydrocarbons were also reported (Johnson and Pecor 1969; Willford et al. 1969). In addition, reduced hatchability of salmon eggs in Sweden was reported as correlated with elevated PCB residues (Jensen et al. 1970). Nevertheless, hatchery records showed that when eggs of planted Lake Michigan lake trout were manually stripped, fertilized, and hatched, and the fry were reared in hatcheries, survival was "normal" or "satisfactory" (Stauffer 1979).

HATCHABILITY OF EGGS

In 1972-73, researchers at the Great Lakes Fishery Laboratory performed studies to investigate further the hatchability of eggs from Lake Michigan lake trout under three sets of incubation conditions: normal hatchery conditions; a thermal regime similar to that of winter and spring in Lake Michigan; and the thermal and chemical conditions characteristic of water from the Hammond Bay Biological Station's intake on Lake Huron. Related studies were carried out by the MDNR at the Marquette State Fish Hatchery, at the Thompson State Fish Hatchery, and at two locations (in egg-holding enclosures) in Lake Michigan's Grand Traverse Bay from 1973 to 1976 (Stauffer 1979). In all of these studies, the survival of contaminated eggs and fry from Lake Michigan lake trout was compared with that of relatively uncontaminated eggs and fry from hatchery brood stock. Although occasional differences in survival were noted between groups of eggs and fry reared under the various experimental conditions, no consistent relation between hatching success and the concentrations of DDTs or PCBs in the eggs was apparent. The conclusion reached in the studies performed at the several locations by the two agencies was that existing levels of DDTs and PCBs in eggs of Lake Michigan lake trout did not significantly affect survival in eggs or of early stages of the fry.

The reproductive failure of lake trout in the lake was nevertheless still apparent in the mid 1970's. We then speculated that although the eggs could hatch and the fry survive in a clean (hatchery or laboratory) environment, the additional chronic exposure to PCBs and DDE in the water and food of Lake Michigan might reduce the stamina, strength, or wariness of the fry sufficiently to preclude their survival in the rigorous lake environment.

SURVIVAL OF FRY

To test this hypothesis, we began a 6-month study in the winter of 1975-76 on the effects of chronic exposure of fry of Lake Michigan lake trout to PCBs and DDE. In addition to routine observations on mortality and growth of the fry, we also evaluated methodology for, and made measurements of, their temperature preference, swimming performance, predator avoidance, and metabolism. About 27,000 eggs were manually stripped and fertilized with milt from lake trout (about 10 females and 20 males) gillnetted in southeastern Lake Michigan near Saugatuck, Michigan in the fall of 1975. Contaminant levels in adult lake trout from this area had been monitored for several years and the fish were known to contain average whole-body concentrations of about 22 μg/g PCBs, 7.5 μg/g total DDT, and 0.3 μg/g dieldrin (Great Lakes Fishery Laboratory, unpublished data). Our analysis of eyed eggs sampled from those collected for this study revealed 7.6 μg/g PCBs and 4.7 μg/g total DDT. Samples of 1-day-old sac fry hatched from these eggs and analyzed at the USFWS Columbia National Fishery Research Laboratory were shown to contain 3.8 μg/g PCBs (Aroclor 1254), 2.3 μg/g total DDT, 0.06 μg/g dieldrin, 0.12 μg/g cis-chlordane, and about 5.7 μg/g of a chemical resembling toxaphene. Later analysis showed that the toxaphene-like residue was actually composed of several chlorinated camphenes of undetermined origin.

The fry were then exposed for 6 months to 10.0 ng/l PCBs (Aroclor 1254) and 1.0 ng/l DDE in water, and 1.0 μg/g PCBs and 0.1 μg/g DDE in food. These values approximate the exposure received by fish in the lake as determined by analyses of water and plankton collected offshore in southeastern Lake Michigan. Concentrations 5 and 25 times these values were also tested to allow dose-effect interpretation and prediction of potential effects on fry hatched in the more contaminated, nearshore areas of the lake.

About a week after the eggs hatched, grossly deformed fry were discarded and the rest were equally divided among 30 tanks (650 fish per tank) in a constant-flow bioassay system. Serial diluters supplied the appropriate concentrations (1x, 5x, 25x, and control) of the contaminants singly or in combination in 9 C well water. The experimental design thus provided for 10 different treatments (including the controls) and three replicates of each. Following 11 days of exposure, the fry began to exhibit feeding behavior and were fed the corresponding dosage of either or both contaminants that had been added to their food. Analyses of water during the study showed that the actual average exposures received by the fry corresponding to 1x, 5x, 25x were 20.8, 64.7, and 327 ng/l PCBs and 1.8, 6.3, and 32.7 ng/l DDE. Analyses of the food showed that actual concentrations were all within 28% of agreement with nominal concentrations.

During the first 16 days of exposure to the three levels of PCBs, DDE, and PCBs plus DDE in water, the percentages of fry that died ranged from 1.9 to 3.7% across all treatments. Mortalities of fry among the nine exposed groups were not significantly different from the percentage that died among the controls. During the next 40 days (days 17-56), when exposed fry began receiving contaminants in their food as well as from the water, the mortality rate in the simulated Lake Michigan exposures (1x) ranged from 2.2 to 3.9%, that in the 5x exposures ranged from 3.5 to 5.9%, and that in the 25x exposures ranged from 7.5 to 24.2%. The mortality rate of control fry (7.3%) was higher during this period than that of fry in the 1x or 5x exposures.

During the second 40-day period (days 57-96), which began about 2 weeks after completion of yolk absorption, mortality of fry increased significantly ($P < 0.01$) in both the exposed and control groups. This increase was most dramatic, however, among the exposed groups of fry. Mortality rates for all nine exposed groups during this period (19.0 to 35.4%) were significantly higher ($P < 0.01$) than in the controls (11.2%). By the end of the third 40-day period (days 97-136), the rates of mortality decreased in all treatments when compared with the previous period but mortality rates in all nine exposed groups (4.5 to 13.4%) nevertheless remained significantly higher ($P < 0.01$) than in the controls (1.3%). Mortality rates further leveled off during the fourth 40-day period (days 137-176), but the final cumulative mortality for each of the nine exposed groups was significantly higher ($P < 0.01$) than that for the controls. The average total cumulative mortality on day 176 in each of the exposed groups ranged from 30.5 to 46.5%, whereas that in the control group was only 21.7%.

Especially noteworthy was the final cumulative mortality of fry in the 1x combination exposure of PCBs and DDE (simulated Lake Michigan exposure)--40.7% or nearly double the final cumulative mortality of the controls (Figure 2). This result suggests that if lake trout in Lake Michigan spawned successfully and their eggs hatched, nearly twice as many of the resulting fry would die within the first 6 months than would have died if these contaminants had not been present. In nearshore areas, where contaminant levels are generally higher, the potential impact on fry mortality would be expected to increase. At the highest combined level of PCBs and DDE tested (25x), 46.5% of the fry died.

PHYSIOLOGY OF FRY

In addition to observations on the mortality of fry during the chronic exposure, observations were made periodically on the growth, swimming performance, predator avoidance, temperature preference, and metabolism of the fry. In general, the exposed fry showed no significant physiological effects attributable to the exposure. Although occasional differences were noted in the swimming performance and in certain metabolic measurements such as oxygen consumption rates and whole-body lactate concentrations after swimming, the results were inconclusive because the variability of the data was high. Procedural difficulties prevented the testing of temperature preference at the 1x and 5x exposures; nevertheless, fry exposed to 25x DDE and 25x DDE and PCBs in combination for 4 months preferred significantly lower ($P < 0.05$) temperatures (9.8 C and 8.7 C, respectively) than did the controls (11.2 C). Because of the inconclusiveness of the observations on the general condition or performance of the fry, together with the inherent difficulty of interpreting the impact of these sublethal effects on the pro-

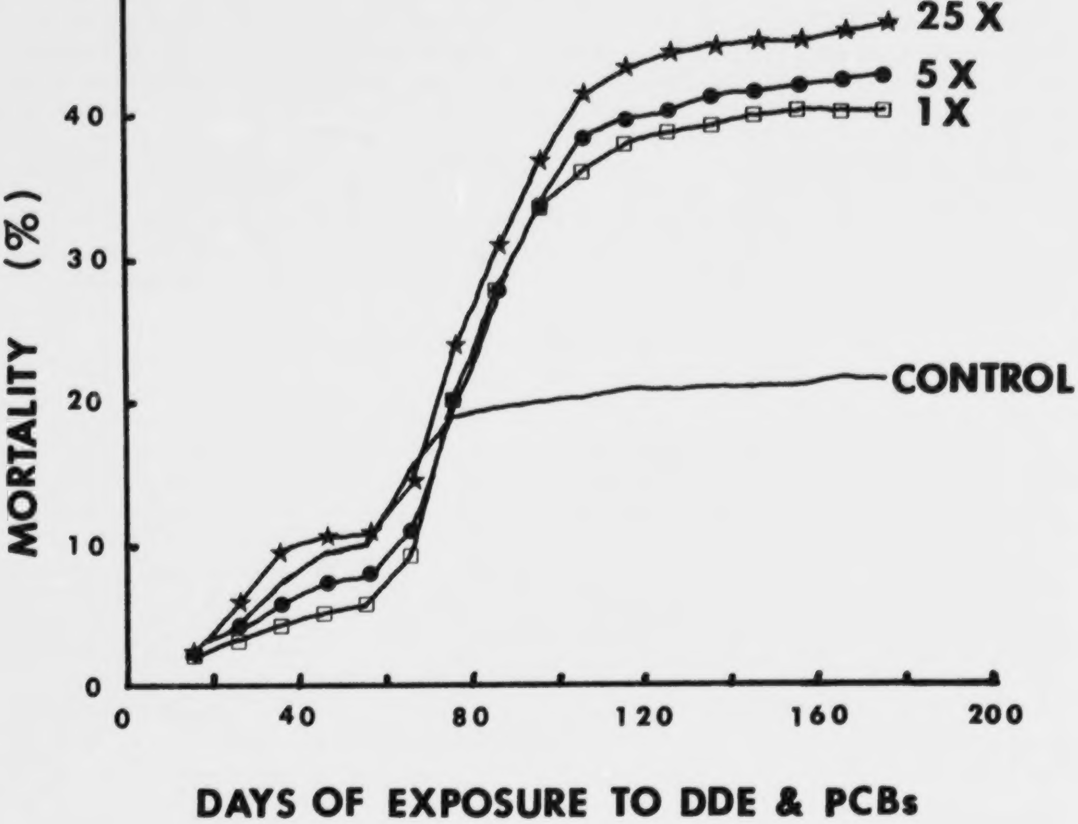

Figure 2. Mortality of fry of Lake Michigan lake trout exposed to DDE and PCBs at concentrations simulating those found in water and plankton of Lake Michigan (1x) and at concentrations 5 and 25 times higher.

ductivity of fish populations, the increase in mortality was clearly the most sensitive and meaningful observation of effect measured in the study.

CONCLUSIONS

The significant increase in mortality of lake trout fry during 6 months of exposure to levels of DDE and PCBs in food and water similar to those in Lake Michigan strongly suggests that these chlorinated hydrocarbons are a limiting factor in the reproduction of lake trout in the lake. Whether these two contaminants are the sole or even major cause for reproductive failure of the lake trout is unclear. Other factors such as the presence of exotic species and the spawning behavior of planted fish undoubtedly play a role. The known presence, however, of additional chlorinated hydrocarbons such as dieldrin, chlordane, and chlorinated camphenes, as well as of several other organic and inorganic contaminants in the water and biota of the lakes, raises serious questions about the potential additive or synergistic effects of these multiple contaminants. Regardless of the ultimate answer to these questions, the current levels of PCBs and DDE in the lake appear sufficient to impede the restoration of self-sustaining populations of lake trout in Lake Michigan.

ACKNOWLEDGEMENTS

The studies and conclusions reported here resulted from the dedicated and professional effort of the entire staff of the Section of Physiology and Contaminant Chemistry, Great Lakes Fishery Laboratory. Special credit goes to Robert E. Reinert for initially identifying chlorinated hydrocarbons as a potential problem in Lake Michigan and for directing the early studies on hatchability of lake trout eggs. Principal investigators in the studies I discussed were William H. Berlin, Roger A. Bergstedt, Robert J. Hesselberg, Michael J. Mac, Dora R. May Passino, and Donald V. Rottiers. The assistance of Lawrence W. Nicholson and James R. Olson in providing chemical analyses for most of the studies, and of Neal R. Foster and Thomas L. Baucom in editing this report is gratefully acknowledged.

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SECTION 7

ORGANOPHOSPHORUS PESTICIDES AND THEIR HAZARDS TO AQUATIC ANIMALS

V.I. Kozlovskaya and B.A. Flerov[1]

Recently, as replacements for DDT and other persistent organochlorine insecticides, a variety of organic phosphorus compounds have been synthesized. At present, world wide utilization of organophosphorus pesticides involves more than 150 compounds (Melnykov, et al. 1977). As a result of their large-scale production and use, this group of toxicants requires investigating.

Pesticides enter the water bodies with the industrial wastes, with the flows from water collectors, with the waters from drainage systems, and from the runoff and overcarriage of the spraying of fields from airplanes.

Organophosphorus pesticides were found in the Kuban River in 7 out of 8 sites examined. Their concentrations varied from 0.04 to 0.3 mg/ℓ (Table 1). In 224 water samples obtained in ponds and rivers of different regions of the Ukraine, organophosphorus compounds were present in 73. Similarly, they were found in 30 out of 216 samples of bottom deposits (Kostovetsky, et al. 1976). In reservoirs of the south and west regions of Slovakia, malathion and sumithion found in amounts of 0.5 - 1 mg/ℓ (Bilikova 1973).

TABLE 1. THE AVERAGE WEIGHT OF ORGANOPHOSPHORUS PESTICIDES AT STATIONS IN THE KUBAN RIVER (1967-1974)

Name of Observation Point	Concentration, mg/ℓ
Karatshayevsk	-
Tsherkassk	0.218
Nevynnomyssk	0.087
Armavir	0.294
Kropotkin	0.246
Krasnodar	0.037
Temryuk (the Petrushkin arm)	0.067
Atshuyevo (the Protok arm)	0.205

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Since organophosphorus pesticides are easily dissolved in water, heavy rains contribute to their intensive runoff from agricultural fields to reservoirs. For example, after a rainfall of 2.1 mm, the phosalon content of the water body located near an orchard treated with this chemical exceeded the permissible concentrations by 7 to 9.6 times, and after a rainfall of 21.1 mm a 12-fold excess was reported (Ivantshenko 1978).

Decomposition of organophosphorus compounds in water compared with organochlorine compounds occurs very rapidly (Table 2). The time of degradation depends on the concentration of hydrogen ions, and temperature (Melnykov, et al. 1977); and it is dependent upon the number of bacteria decomposing these compounds (Ulyanova, et al. 1979).

TABLE 2. PERSISTENCE OF SELECTED ORGANOPHOSPHORUS PESTICIDES IN WATER

Pesticide Type	Concentration mg/ℓ	Period of Complete disappearance in days	Reference
Metaphos	0.02	3-5	Kostovetsky, et al. 1976
	0.2	8-14	Kostovetsky, et al. 1976
	1-2	55	Ulyanova, et al. 1979
	2.5	160	Ulyanova, et al. 1979
Dylox	0.05	1	Kostovetsky, et al. 1976
	0.5	10	Kostovetsky, et al. 1976
Malathion	0.1	14	Drevenkar, et al. 1975
	0.5	6-11	Kostovetsky, et al. 1976
Bazudin	0.6	16	Boyko and Pulatov, 1977
	6.0	21	Boyko and Pulatov, 1977
	60.0	35	Boyko and Pulatov, 1977
DDVP	0.1	11	Drevenkar, et al., 1975

Both the intensity and duration of effects upon water bodies are primarily determined by the length of time that pesticides stay in the soil of catchment areas. Depending on the type of soil, humidity, and pH, pesticides may be retained for extended periods of time and with surface water flows, enter reservoirs (Table 3).

Organophosphorus pesticides in concentrations most commonly found in water bodies, show a high toxicity for aquatic animals, especially for planktonic invertebrates and aquatic insects (Table 4). In 48-hour exposures to 0.001 mg/ℓ solutions of malathion, Simocephalus vetulus became less mobile and died after being placed in freshwater for recovery. The 48-hour LC_{50} for the eggs of carp is approximately 0.01 mg/ℓ, but for their larvae the value is ten times as high (Prokopenko, et al. 1976). Eight day larval forms of freshwater invertebrates demonstrate depression changes after three

TABLE 3. PERSISTENCE OF SELECTED ORGANIC PESTICIDES IN SOIL

Pesticides	Period of complete disappearance in days	Reference
Metaphos	10-150	Korotova and Demtshenko, 1978a Kostovetsky, *et al*., 1976 Yurovskaya and Jhulinskaya, 1974
Dylox	4-45	Korotova and Demtshenko, 1978b Kostovetsky, *et al*., 1976 Yurovskaya and Jhulinskaya, 1974
Malathion	7-60	Kostovetsky, *et al*., 1976 Keazney, *et al*., 1969 Novozhylov, *et al*., 1974
Diazinon	85	Keazney, *et al*., 1969 Takase, 1976
Phosalon	18-90	Manko, *et al*, 1974

TABLE 4. TOXICITY OF ORGANOPHOSPHORUS PESTICIDES TO AQUATIC ANIMALS
(From Water Quality Criteria, 1972, EPA-R-73-033, 1973)

Animal species	96-hour LC , mg/ℓ			
	Guthion	Malathion	Parathion	Dylox
Gammarus lacustris	0.00015	0.001	0.0035	0.04
Gammarus fasciatus	0.0001	0.00076	0.0021	-
Asellus brevicaudus	0.021	3	0.6	-
Daphnia pulex	-	0.0018	0.0006	0.00018
Pteronarcys dorsata	0.0121	-	0.003	-
Pteronarcys californica	0.0015	0.01	0.036	0.069
Acroneuria lycorias	-	0.001	-	-
Acroneuria pacifica	-	-	0.003	0.0165
Salmo gairdneri	0.014	0.17	-	-
Salmo trutta	0.004	0.2	-	-
Oncorhynchus kisutch	0.017	0.101	-	-
Lepomis macrochirus	0.0052	0.11	0.065	3.8
Pimephales promelas	0.093	9	1.41	109

exposures to malathion in concentrations of 0.002 and 0.02 mg/ℓ. Chironomid and mayflies also decrease considerably (Kennedy and Walsh 1970).

Lesnikov (1974) suggests that the most sensitive indicator of the effects produced by organophosphorus compounds is an increase of both population and biomass of aquatic organisms (Table 5).

TABLE 5. DYLOX TOXICITY (mg/ℓ) FOR SELECTED AQUATIC ORGANISMS

Animal species	Toxicity Acute	Toxicity Chronical	Effect on the increase of biomass
Daphnia longispina	0.0005	0.0001	0.00002
Gammarus pulex	0.5	0.1	0.03
Salmo irideus	0.121	0.06	0.004

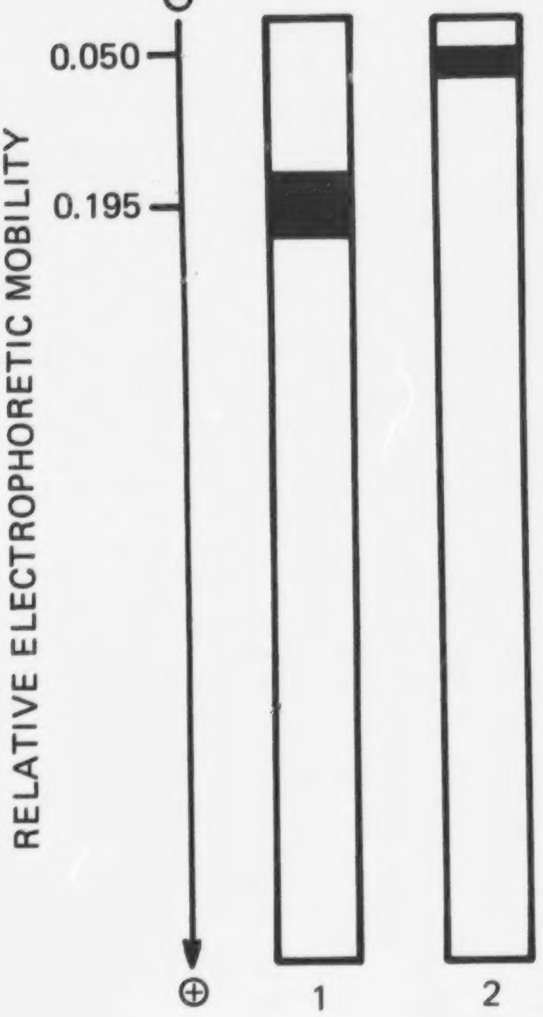
The hazards of organophosphorus pesticides are even greater since animals demonstrate poor avoidance reactions to these chemicals. Some invertebrates do not avoid Dylox at all (*Hirudo medicinalis*), or some such as *Asellus aquaticus* and *Stephocephalus torvicornis* avoid it only in concentrations of 250 to 1000 times higher than their 48-hour LC_{50} (Flerov and Lapkina 1976; Tagunov and Flerov 1978; Flerov and Tagunov 1978). Guppies demonstrate avoidance reactions to Dylox at concentrations equal to the 48-hour LC_{100} (Flerov 1979).

Shrimp (*Palaemonetes pugio*) fail to avoid malathion (Hansen, *et al*. 1973), and mosquito fish avoid it only in acutely toxic concentrations (Hansen, *et al*. 1972).

The toxicity of organophosphorus compounds is attributed to their ability to inhibit acetyl cholinesterase irreversibly, which in turn, depends upon the particular enzyme system in the animals.

Thus, the two species of gastropods (*Limnaea stagnalis* and *Planorbis corneus*) differ in resistance to Dylox by 100 times. The nervous ganglia of these forms contain enzymes of the acetyl cholinesterase type, that hydrolyze the same substrates, but differ in quantity, electrophometic mobility and sensitivity to the toxicant. *In vitro* experimentation with the sensitive species (*Limnaea stagnalis*) showed concentrations of 10^{-2} to 10^{-4}M Dylox completely inhibited enzyme activity, 10^{-5}M inhibited by 97 percent, and 10^{-6}M inhibited by 61 percent. In the resistant species, *Planorbis corneus*, even concentration of 10^{-4}M of toxicant did not inhibit the enzyme completely, although the enzyme content in ganglia of this species is much lower than in *Limnaea stagnalis* (Figure 1 and 2).

The correlation between the resistance of organism to the toxicant and the sensitivity of an enzyme to it has also been observed in fish. The roach, *Rutilus rutilus*, and the blue bream, *Abramis ballerus*, are poorly resistant to Dylox. Their blood sera contains an enzyme of the cholinesterase type which is absent in more resistant fish, such as the carp, *Cyprinus*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	Molusc species	48-Hour LC_{50}, mg/l
1	*L. stagnalis*	0.5
2	*P. corneus*	50.0

Figure 1. Acetycholinesterase in nervous ganglia of molluscs with varying resistance to Dylox.

1 - *Limnaea stagnalis*, LC_{50} - 0.5 mg/l 48-hrs. exposure,

2 - *Planorbis corneus*, LC_{50} - 50 mg/l 48-hrs. exposure.

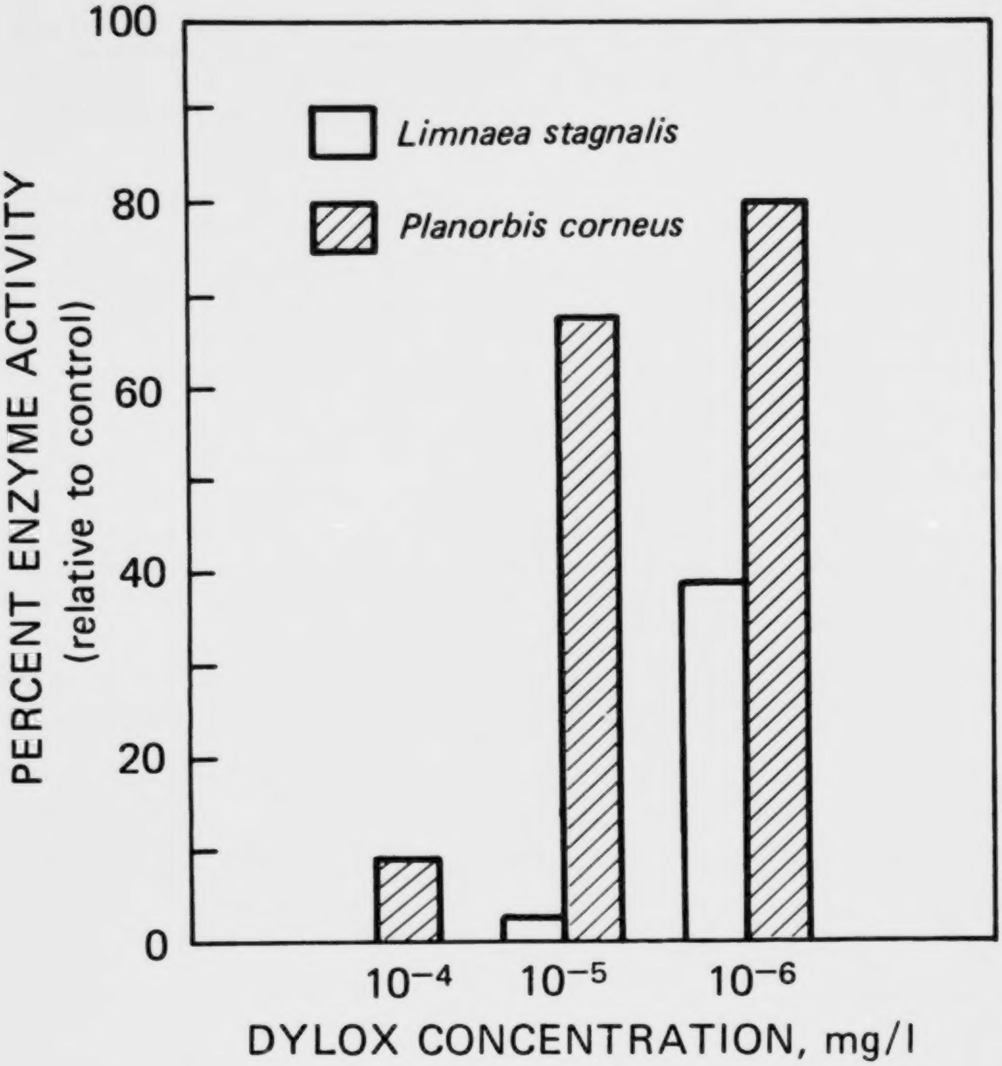

Figure 2. Inhibition by Dylox of acetycholinesterase in nervous ganglia of Limnaea stagnalis and Planorbis corneus.

carpio and the bream, *Abramis brama*. The latter contains an enzyme of the acetyl cholinesterase type (Kozlovskaya and Tshuyko 1979).

As intoxication by organophosphorus pesticides advances, the animals exhibit a progressive decline in the level of cholinesterase, although in dying animals the enzyme may not be entirely inhibited. Such facts are cited in a number of reviews (O'Brian 1964; Rosengart and Sherstobitov 1978).

After acute exposure of perch (*Perca fluviatilis*) to Dylox (48-hour LC_{100} of 5 mg/ ; 48-hour LC_{50} of 0.62 mg/ℓ) fish were assayed immediately after death, 8 and 33 hours of the experiment, respectively. The cholinesterase activity in these cases was partially retained (up to 25 percent). In fish which were left in the toxic environment after death for a few hours, the enzyme was inhibited to a greater extent (Figures 3a and 3b). Similar results were obtained in experiments with carp (*Carassius carassius*) and pond snails (*Limnaea stagnalis*). Densitometry of electrophorograms showed that not all molecular forms of the enzyme were completely inactivated (Figures 4a and 4b). It appears that the toxicant interacts with vitally important forms of the enzymes.

The inhibitation of AChE in the brain of perch has been also observed at sublethal concentrations, although the external symptoms of poisoning were absent (Table 6). Upon placing the animals in freshwater, the gradual reactivation of enzymes took place.

TABLE 6. CHANGES IN THE ACETYL CHOLINESTERASE ACTIVITY OF THE PERCH BRAIN IN THE MINIMUM TOLERABLE CONCENTRATIONS OF DYLOX (0.12 mg/ℓ) WITH SUBSEQUENT WASHING IN FRESHWATER

		Enzyme of Activity	
Exposure	Number of samples	μM AChE g/h	% of the control
Exposure in the Dylox Solution	1-10	427.9 ± 0.84	87.2
	5-10	339.3 ± 0.79	67.6
One day exposure in freshwater after 5-days exposure in Dylox	1-15	358.6 ± 1.03	73.5
	5-11	507.1 ± 0.43	97.4

The periodic addition of Dylox to the test system causes increases in inhibition of AChE with each dose. Fish mortality occurs at a total concentration of 0.36 mg/ℓ, considerably below the minimum lethal concentration (Table 7).

Similar results have been obtained with experiments on roach. Daily exposure to one-tenth of the 48-hour LC_{100} led to a greater toxic effect than the exposure in concentrations equal to the full 48-hour LC_{100}.

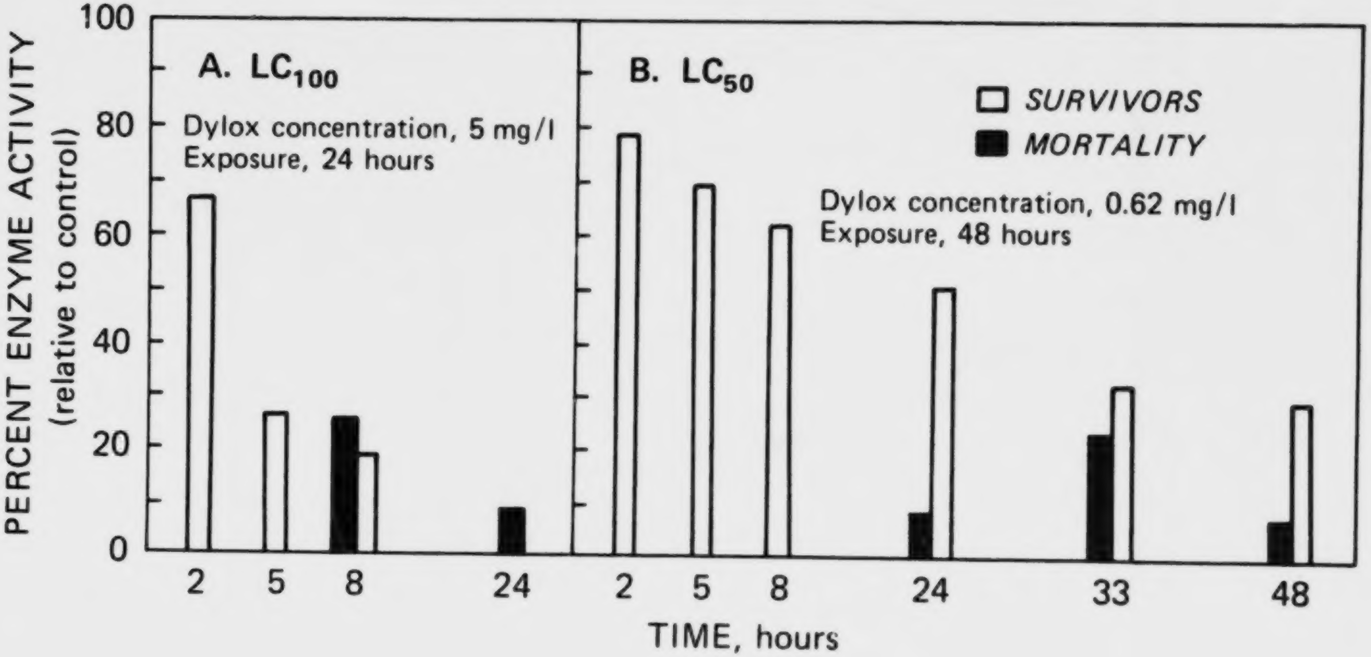

Figure 3. Change in the activity of acetylcholinesterase in perch (Perca fluviatilis) brain after exposure to Dylox.

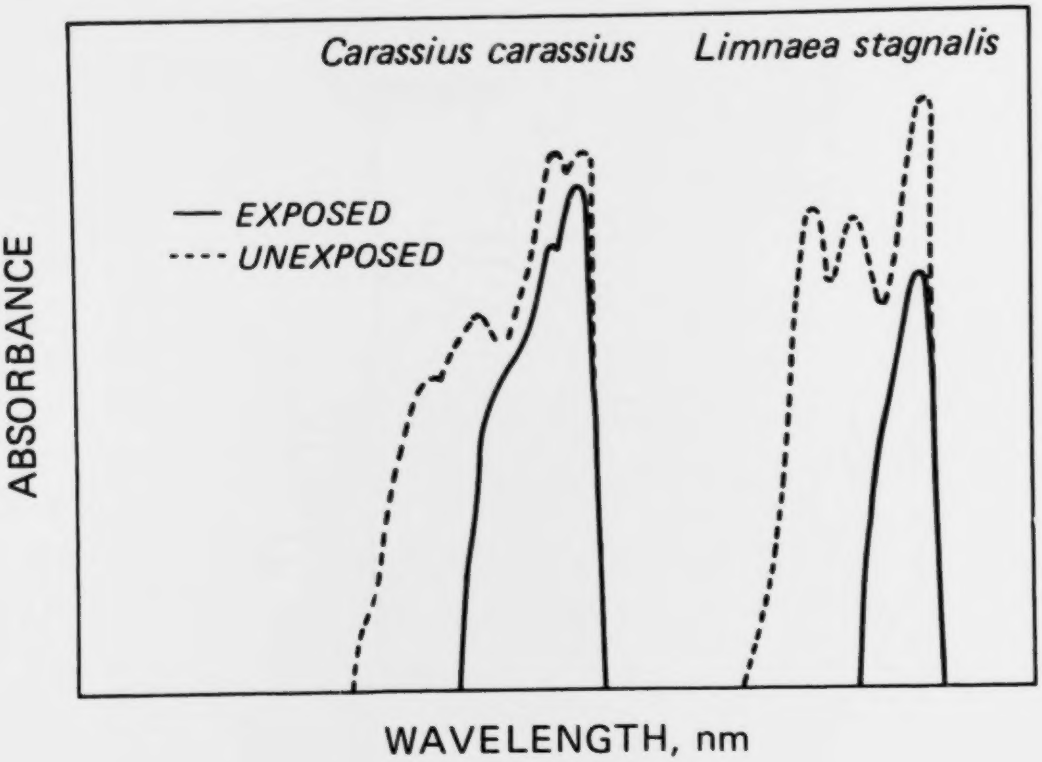

Figure 4. Densitograms of the molecular form of acetylcholinesterase in carp (Carassius carassius, 1) and the snail (Limnaea stagnalis, 2) unexposed and exposed to Dylox.

TABLE 7. CHOLINESTERASE ACTIVITY IN PERCH BRAIN AS A RESULT OF PERIODIC ADDITIONS OF DYLOX TO THE EXPOSURE CHAMBER

Days of observation	Concentration mg/ℓ	Enzyme Activity			Percent mortality
		Number of samples	μM acetyl choline gm/hr	Percent of the control fishes	
1	0.12	-	-	-	0
5	0.12	10	339.3 ± 0.94	67.6	0
10	0.12	10	207.7 ± 0.42	42.3	2
11	-	-	-	-	15
12	-	10	147.6 ± 0.64	31.4	52
13	-	9	140.2 ± 0.58	28.5	76
14	-	-	-	-	94
15	-	-	-	-	100

Cholinesterase has been inhibited more in the first case (Kozlovskaya and Novichikova 1979). Organophosphorus pesticides on continued chronical exposure prove no less dangerous than with acute intoxication.

CONCLUSIONS

Organochlorine pesticides have been replaced with organophosphorus on the assumption that as a result of lower persistence in the aquatic environment those compounds will be of little danger to aquatic organisms. Organophosphorus pesticides have proven to be highly toxic to the majority of species of aquatic invertebrates. The data provided in this study demonstrates that there are concentrations in reservoirs, which greatly exceed lethal levels for sensitive species.

The intensive application of organophosphorus pesticides as a part of agricultural practices results in a periodic influx of these pesticides into water bodies. In natural waters, pollution levels are produced which cause chronic effects upon aquatic animals. This is especially dangerous because organophosphorus compounds possess an additive effect, and are poorly avoided by aquatic animals.

An indicator of the effects of organophosphorus pesticides in an inhibition of cholinesterase in both acute and chronic intoxication. In pathological processes, the inhibition of cholinesterase as a target enzyme undoubtedly plays a leading role, although death occurs when the inhibition of enzymes is still incomplete.

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SECTION 8

MONITORING CONTAMINANT RESIDUES IN FRESHWATER FISHES IN THE UNITED STATES: THE NATIONAL PESTICIDE MONITORING PROGRAM

J. Larry Ludke and C.J. Schmitt[1]

INTRODUCTION

The National Pesticide Monitoring Program (NPMP) originated in the mid 1960's as a cooperative effort by members of national agencies of the Federal Committee on Pest Control. In 1972 the overall responsibility for NPMP activities was given to the United States Environmental Protection Agency (EPA). EPA then developed a comprehensive National Monitoring Plan for Pesticides, which describes and sets broad guidelines for various other federal agencies cooperating in monitoring pesticide trends in soil, water, air, man, plants and animals (Table 1). Each participating agency monitors chemical residues in the one or more segments of the environment which it is charged with protecting or regulating. In recent years chemical contaminants other than pesticides, such as polychlorinated biphenyls (PCBs) have been added to the list of chemical residues that are routinely analyzed.

For the purposes of the NPMP, monitoring can be defined as the repetitive observation of one or more segments of the environment according to a prearranged schedule in space and time. The overriding objective of the NPMP is to ascertain on a nationwide basis, the levels and temporal trends of selected contaminants in the environment.

A secondary objective of the NPMP is to identify areas where unusually high residues may occur (i.e., problem areas) and which therefore may require more intensive study to determine potential contaminant sources and possible detrimental effects. Data may also be used to initiate or evaluate management and regulatory actions.

U.S. FISH AND WILDLIFE SERVICE SUBPROGRAMS

The U.S. Fish and Wildlife Service is responsible for the fish and wildlife subprogram of the NPMP, the primary objective of which is to ascertain

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TABLE 1. NATIONAL PESTICIDE MONITORING PROGRAM NETWORK: A LIST OF ENVIRONMENTAL COMPONENTS AND THE RESPECTIVE AGENCIES RESPONSIBLE FOR MONITORING CONTAMINANT TRENDS IN EACH

Environmental Component	Agencies
Soils	Environmental Protection Agency (EPA)
Water and Sediment	Environmental Protection Agency U.S. Geological Survey (USGS)
Oceans, Bays, and Estuaries Marine Fauna	National Oceanic and Atmospheric Agency (NOAA) Public Health Service
Atmosphere (pilot program)	Environmental Protection Agency
Avian Wildlife	U.S. Fish and Wildlife Service (FWS)
Freshwater Fishes	U.S. Fish and Wildlife Service
Food and Feed	U.S. Department of Agriculture (USDA) Food and Drug Administration (FDA)

on a nationwide basis, and independent of specific treatments, the levels and trends of selected environmental contaminants in freshwater fishes and selected bird species. In addition to monitoring trends in contaminants, the Fish and Wildlife Service also investigates the sources and impacts of contaminants on natural resources. The Columbia National Fisheries Research Laboratory (CNFRL) is responsible for monitoring residue trends in freshwater fishes and Patuxent Wildlife Research Center, Laurel, Maryland is responsible for monitoring residues in tissues of selected waterfowl and starlings (*Sturnus vulgaris*).

FRESHWATER FISH FROM LAKES AND STREAMS

Monitoring contaminants in freshwater fish has undergone a series of changes since collections began in 1967. At first, fish were collected from 50 sampling stations in the Great Lakes and major rivers throughout the United States (Stations 1-50, Figure 1). Five adult fish of each of three predominant species were collected in the spring and again in the fall of both 1967 and 1968. In 1969, and each year since then, collections have been made only in the fall. In 1970 the number of collection stations was increased to 100 with the addition of Stations 51-100 (Figure 1). Determinations have always been based on composited, whole-body samples of five fish each. From 1967 through 1971 all sample analyses were contracted to a private laboratory; in 1972, for economic and administrative reasons, the analytical work was shifted to the Fish and Wildlife Service Laboratory in Denver, Colorado; and in 1976 the program was relocated to CNFRL, where it remains today. Collections were suspended for one year in 1975 when freshwater fish monitoring was undergoing an internal review and reorganization.

There are now 117 stations in the United States where fish are collected for analysis of contaminant residues (Figure 1). About half of the stations are sampled in the Fall of even-numbered years and the other half during odd-numbered years. At each trend monitoring station three samples of five fish each are taken: two samples of a predominant bottom-dwelling species and one sample of a predator species. The preferred species to be collected vary geographically and according to habitat (Table 2).

The number of contaminants studied has increased over the years from eight in 1967 to more than 20 today (Table 3). At CNFRL there is a strong research emphasis on improving methods and developing the technology necessary to quantify toxic chemical contaminants that are difficult to analyze in biological tissues.

PROCEDURES

Fish are collected by non-chemical means (i.e., by electroshocking, netting or hook and line) according to specified instructions. Sometimes fish must be purchased from local commercial fishermen known to fish in the vicinity of the collection site. All specimens are adult fish, preferably of uniform size, and weighing no more than 22.7 kg (5 lb) each.

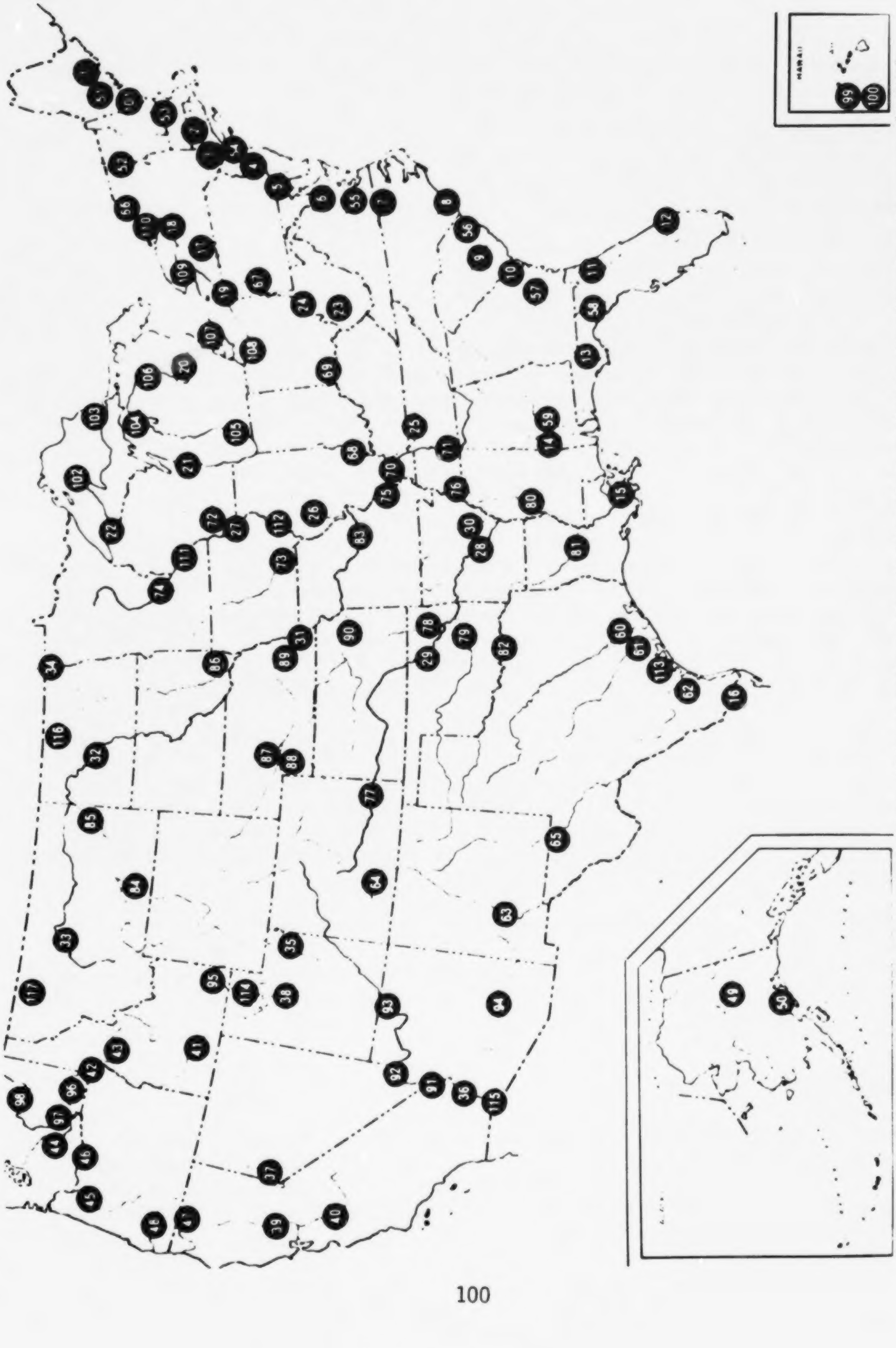
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Figure 1. Map of the United States illustrating the National Pesticide Monitoring Program (NPMP) stations where freshwater fish are collected for routine contaminants analyses.

TABLE 2. FRESHWATER FISHES RECOMMENDED FOR COLLECTION FOR TISSUE CONTAMINANT RESIDUE DETERMINATIONS (NPMP), LISTED BY CATEGORY, HABITAT AND (IN THE ORDER OF PREFERENCE) SPECIES

Category of fish, habitat, and species[1]
Predator
Cold water
Rainbow trout, *Salmo gairdneri*
Brown trout, *S. trutta*
Brook trout, *Salvelinus fontinalis*
Lake trout, *S. namaycush*
Cool water
Walleye, *Stizostedion vitreum*
Yellow perch, *Perca flavescens*
Sauger, *S. canadense*
Northern pike, *Esox lucius*
White perch, *Roccus americanus*
Other percid (Percidae) or temperate bass (Perichthyidae)
Warm water
Largemouth bass, *Micropterus salmoides*
Other sunfish (Centrarchidae)
Bottom Dwelling
All habitats
Carp (*Cyprinius carpio*)
Channel catfish (*Ictalurus punctatus*)
White sucker (*Catostomus commersoni*)
Other locally abundant sucker (Catostomidae) or catfish (Ictaluridae)

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[1] Predator species are listed in order of preference for each habitat; order of preference for bottom dwelling species is the same for all habitats.

TABLE 3. CONTAMINANT RESIDUES MEASURED AND DETECTED IN NPMP FRESHWATER FISH SAMPLES, 1967 THROUGH 1976-77

	Year								
Contaminant	1967	1968	1969	1970	1971	1972	1973	1974	1976-1977
p,p' - DDE	+[1]	+	+	+	+	+	+	+	+
p,p' - DDD	+	+	+	+	+	+	+	+	+
p,p' - DDT	+	+	+	+	+	+	+	+	+
o,p' - DDE	NA[1]	NA	NA	+	+	+	+	+	+
o,p' - DDD	NA	NA	NA	+	+	+	+	+	+
o,p' - DDT	NA	NA	NA	+	+	+	+	+	+
Aroclor 1242	NA	NA	NA	NA	NA	-[1]	-	+	+
Aroclor 1248	NA	NA	NA	NA	NA	NA	NA	+	+
Aroclor 1254[2]	NA	NA	+	+	+	+	+	+	+
Aroclor 1260	NA	NA	NA	NA	NA	+	+	+	+
Aldrin & dieldrin	+	+	+	+	+	+	+	+	+
Endrin	+	+	-	+	+	+	+	+	+
Lindane[3]	+	+	NA	NA	NA	NA	NA	NA	+
α-benzene hexachloride (α-BHC)[4]	NA	NA	+	+	+	+	+	+	+
Heptachlor & heptachlor epoxide	+	+	+	+	+	+	+	+	+
Chlordane	+	+	+	+	+	+	+	+	+
Toxaphene	NA	NA	NA	NA	+	+	+	+	+
Hexachlorobenzene (HCB)	NA	NA	NA	NA	+	+	+	+	+
Arsenic									+
Selenium									+
Mercury									+
Lead									+
Zinc									+

[1] On the body of the table, + indicates that the contaminant was detected in at least one sample and - indicates that none was detected. NA = not analyzed.

[2] Total PCB as Aroclor 1254, 1969-1971.

[3] Lindane (γ - benzene hexachloride) separated beginning 1976.

[4] BHC as technical, 1969-74; as α - BHC beginning 1976.

Five fish (no fewer than three) are pooled to make up a sample and no sample may exceed 113.4 kg (25 lb). Fish are rinsed in tap water and care is taken to insure that they do not come into contact with potential contaminating surfaces such as plastics, printed paper, metal, or mud. Each fish is weighed and measured (total length) and the age of each fish is determined whenever possible. Fish are then wrapped individually in clean aluminum foil and labeled, after which the specimens making up each pooled sample are placed into a heavy bag and frozen immediately in dry ice. The samples are then transported frozen, by air freight, to CNFRL for analysis.

Fish samples are kept frozen until the time of analysis. The five specimens are then thawed, homogenized and appropriate subsamples are removed for analysis of metals or chlorinated organic contaminant residues. Metals are analyzed by atomic absorption spectrometry, and organochlorine compounds are measured by gas-liquid chromatography; organic residues in some samples are confirmed using mass spectrometry. Selected samples are sent to an independent laboratory for analysis as a means of confirming results.

SELECTED TEMPORAL AND GEOGRAPHIC TRENDS IN CONTAMINANT RESIDUES

Residues of DDT and it metabolites in fishes from the nation's major rivers and lakes have shown a continuing downward trend. The steady decrease in total DDT, as reflected in summed p,p'-homologues (Figure 2) illustrates the effectiveness of the 1972 ban on the use of DDT in the United States. Although DDT residues remain high in some areas where it was used extensively in the past, the overall trend has been downward. Even in those areas where total DDT residues remain high, the p-p'-homologue, DDE, is present in much greater proportion than in the past (Table 4), indicating substantial degradation of DDT and DDD in the environment.

The number of collection sites where DDT has been observed in at least one samples has also decreased somewhat since 1970 (Table 5). Although the present occurrence of p,p'-DDT appears to have increased in recent years (1976-77), this change can probably be attributed to improved analytical techniques that enable better resolution and higher sensitivity for organochlorine contaminants.

PCBs have become virtually ubiquitous, reflecting the former widespread use of these persistent industrial compounds as hydraulic fluids and as heat transfer agents in capacitors and other electrical equipment. Fish containing residues of 0.5 μg/g (wet weight, whole fish), the criterion established for the protection of piscivorous fishes and wildlife, are collected regularly from all NPMP stations near urban and/or industrial areas, and trace levels are present in fish from the major watershed of all 50 states.

Definite trends in the overall magnitude of PCB residues are more difficult to discern due to the evolution of analytical methods between 1970 and 1974 (Tables 3 and 4). While there appears to be a slight downward trend nationwide, especially in Aroclor 1254 residues, more data produced by

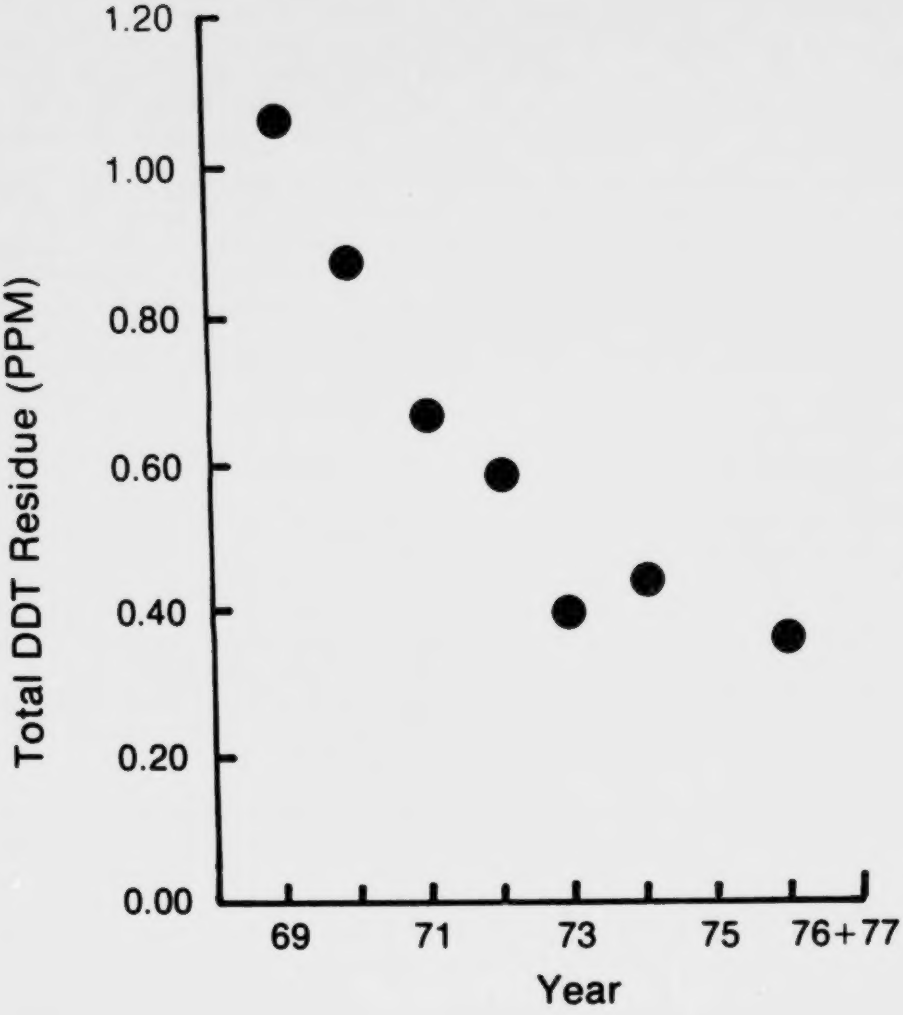

Figure 2. Geometric mean total DDT residues (p,p' - homologues) in freshwater fish, 1969-1976/77.

TABLE 4. GEOMETRIC MEAN RESIDUES OF ORGANOCHLORINE COMPOUNDS AT 74 SELECTED NPMP STATIONS, 1970-1976/77

	Year					
Compound	1970	1971	1972	1973	1974	1976-77
p,p'-DDT	0.27	0.19	0.11	0.07	0.05	0.05
p,p'-DDD	0.34	0.25	0.18	0.12	0.14	0.08
p,p'-DDE	0.47	0.35	0.40	0.30	0.37	0.24
Total DDT	0.98	0.73	0.64	0.44	0.52	0.35
Aroclor 1254	1.20	1.03	1.21	0.58	0.82	0.49
Total PCB	1.20[2]	1.03[2]	1.21[2]	0.78[3]	0.95[4]	0.87[4]
Toxaphene	NA[5]	0.01[6]	0.13	0.17	0.17	0.36
Aldrin + Dieldrin	0.08	0.07	0.07	0.05	0.09	0.06
Endrin	0.01	0.02	0.01	0.01	0.01	0.01

[1] p,p'-homologues

[2] As Aroclor 1254

[3] Aroclor 1242 + 1254 + 1260

[4] Aroclor 1242 + 1248 + 1254 + 1260

[5] Not analyzed

[6] Not analyzed

TABLE 5. PERCENTAGE OF 74 NPMP STATIONS WHERE DETECTABLE RESIDUES OF IMPORTANT ORGANOCHLORINE COMPOUNDS WERE FOUND, 1970-1976/77

Compound	Year					
	1970	1971	1972	1973	1974	1976-77
p,p'-DDT	100	98.6	74.3	41.9	48.6	87.8
p,p'-DDD	100	98.6	97.3	71.6	78.4	100
p,p'-DDE	100	98.6	97.3	95.9	95.9	100
Total DDT[1]	100	98.6	100	100	97.3	100
Total PCB	98.6[2]	98.6[2]	83.8[2]	70.3[3]	93.2[4]	91.9[4]
Toxaphene	NA[5]	13.5	9.5	12.2	14.9	60.8
Aldrin + Dieldrin	100	100	81.1	70.3	52.7	95.9
Endrin	31.1	82.4	10.8	20.3	2.7	48.6

[1] p,p'-homologues

[2] As Aroclor 1254

[3] Aroclor 1242 + 1254 + 1260

[4] Aroclor 1242 + 1248 + 1254 + 1260

[5] Not analyzed

today's methods are needed to substantiate this trend. However, residues at the most heavily contaminated sites appear to be declining more noticeably.

PCBs occur in fish tissues most frequently and at the highest concentrations in the industrial northeastern and midwestern sections of the United States (Figure 3). Though no longer manufactured in the United States, PCBs are still used and continue to contaminate the environment as a result of spills and improper disposal of waste hydraulic fluids and discarded electrical components.

Mean toxaphene residues are increasing in freshwater fishes of the United States (Table 4). The national geometric average has increased from 0.13 μg/g in 1972 to 0.36 μg/g in 1976-77, and residues exceeding 1.0 μg/g are not uncommon. Studies by CNFRL have shown that toxaphene residues of 1 μg/g may be associated with impaired growth and developmental abnormalities in young fish.

Toxaphene also occurs much more widely now than it did in past years (Table 5). Formerly found only in fish from the cotton growing regions of the Southeast and Southwest, it now occurs in fish throughout the United States (Figure 4). Its growing ubiquity may be explained by the increased use of toxaphene in agriculture, largely as a substitute for DDT and other compounds that have been banned. However, this interpretation is complicated by findings indicating the possible occurrence of chlorinated camphenes that behave like certain toxaphene components during gas chromatographic analysis. Particularly high residues of this compound have been found in fishes from the Upper Great Lakes. Despite extensive investigation by gas-liquid chromatography and mass spectrometry, neither the identity nor the source of this compound has yet been satisfactorily determined.

Nationally, average residue of dieldrin and endrin in fish tissues have remained essentially unchanged from 1970 through 1977 (Table 4). Dieldrin residues remained widespread (Table 5), reflecting the extensive use of this compound (and aldrin) before 1974. The apparent variation in endrin occurrence (Table 5), however, may merely indicate changing analytical resolution; endrin residues have remained generally low (Table 4).

Using newly developed capabilities to measure trace metals, we at CNFRL analyzed the fish samples collected in 1977 (representing 54 stations) for residues of Cd, Pb, Hg, As, and Se. 'Background' levels for the five metals in whole fish samples was determined, as well as geographic areas where these levels are exceeded. As examples, we found As levels ≥.5 μg/g in fish from Texas, Oklahoma, and the Upper Great Lakes; Se of ≥1.0 μg/g at many stations in the Upper Missouri River system, and at both stations in Pennsylvania; Pb ≥1.0 μg/g at a group of stations in the central Missouri River system; Hg ≥0.25 μg/g in the Great Lakes and in some Gulf Coast rivers; and Cd ≥0.15 μg/g at two Upper Missouri stations.

Discerning geographic and temporal trends in contaminant residues is not the only result of NPMP monitoring activities. More importantly, the results of these efforts are reflected in the planning of research at CNFRL. For example, unknown gas chromatograph peaks are resolved using mass spec-

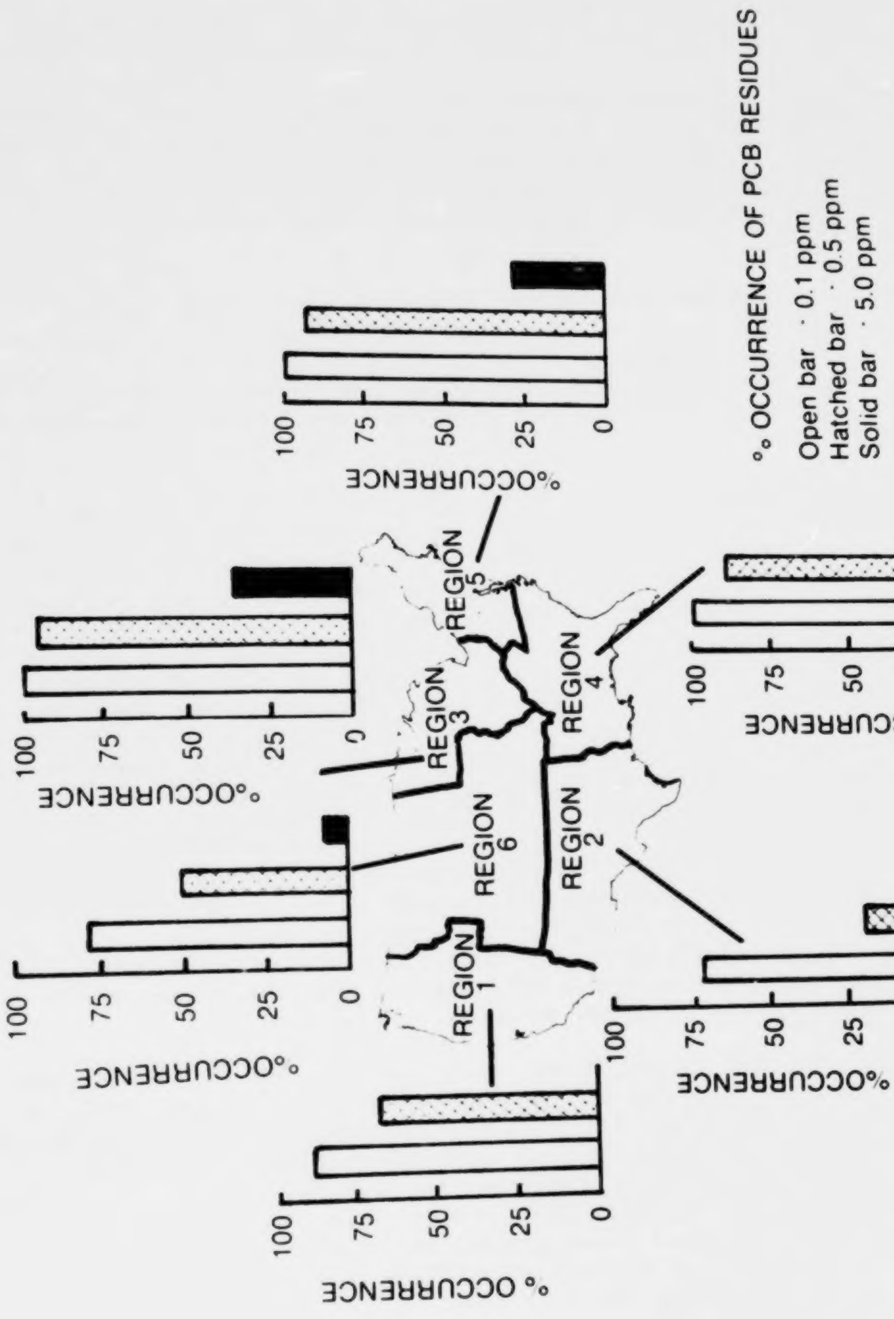

Figure 3. Percent occurrence of polychlorinated biphenyl (PCB) residues in freshwater fish, by U.S. Fish and Wildlife Service Region, 1976/77.

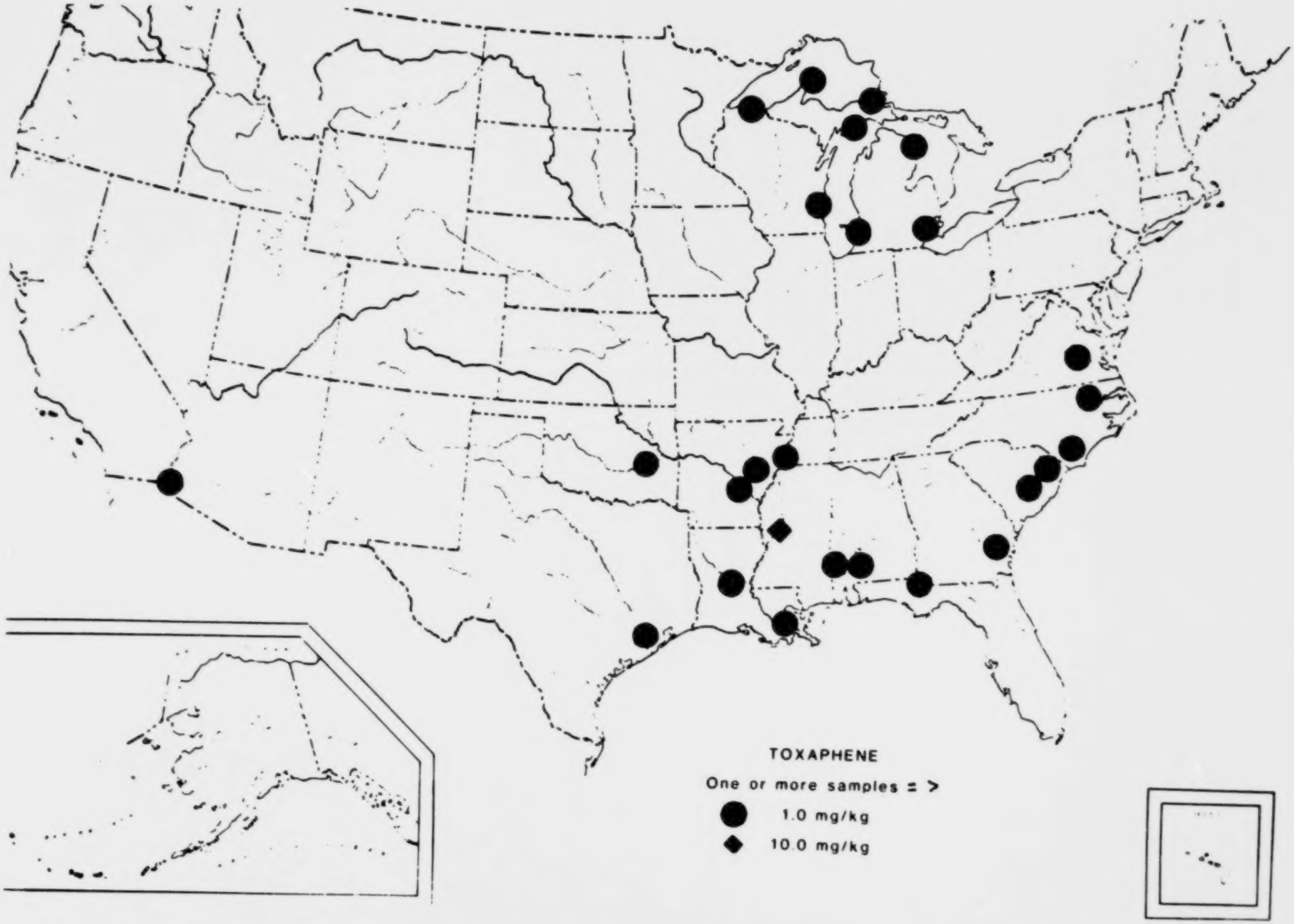
TOXAPHENE
One or more samples = >
● 1.0 mg/kg
◆ 10.0 mg/kg

Figure 4. Occurrence of toxaphene residues exceeding 1.0 mg/kg in freshwater fish (1976-1977).

tral analysis, which in turn may generate a list of candidate compounds for toxicity testing. Or, the consistent occurrence of a given compound from one location may stimulate a cooperative effort with Fish and Wildlife Service Regional personnel, as in the cases of recent investigations of DDT in the lower Rio Grande and toxaphene in the Great Lakes, to determine the source and magnitude of the regional problem. And finally, questions arising from the analysis of NPMP samples continue to stimulate the development of new analytical approaches.

SECTION 9

ACCUMULATION AND METABOLISM OF PERSISTENT PESTICIDES IN FRESHWATER FISH

F.Ya. Komarovskiy and A.Ya. Malyarevskaya[1]

Long term utilization of persistent organochlorine pesticides, especially DDT, and BHC on a world wide basis has led to their distribution and accumulation in a wide variety of media including soil, water, sediments, and aquatic organisms. The accumulation of persistent pesticide residues in organs of aquatic species and their tendency to be transformed in trophic food chains are additional factors aggravating the danger of water pollution by pesticides, both for regeneration of the biological resources of aquatic ecosystems and for the health of man using fish for food.

Studies of the last decade indicated the possibility of understanding the fundamental principles of DDT distribution in the biosphere, including the world ocean; its accumulation in the biota; the role of DDT in ecosystems of different types; demonstrated the biological danger of DDT residues for animals and man; and established the mechanism of its metabolism in abiotic media and in aquatic organisms. While our knowledge has increased and information on the subsequent biological effects of wide-scale DDT utilization has increased, a great number of unsolved problems requiring further research remain. For example, comparatively little data are available on DDT accumulation in brain tissue of warm-blooded animals and fish, even though the neurophilicity of the compound suggests that it should have received the greatest attention. There are very few studies available which show that the development of clinical symptoms of intoxication in warm-blooded animals correlates with an increase of DDT accumulation in brain tissue (Hayden 1960).

One of the most important principles of the biotic circulation of organochlorine pesticides, especially DDT, is their accumulation and transformation in trophic chains, and their tendency to concentrate in the highest links of these chains. This phenomenon is well demonstrated in studies of terrestrial and marine ecosystems (Mayer-Bode 1966; Andryuschchenko and Pishcholka 1975), but has received little attention in freshwater ecosystems.

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Recently, the processes of organochlorine pesticide accumulation in trophic chains have been experimentally modeled to obtain more detailed information on the transformation mechanism in ecosystems. Metcalf, *et al*. (1971) used this experimental approach to select organochlorine pesticides with lowest accumulation factors, i.e., those which were poorly accumulated, and which were not transformed in trophic chains.

The question of metabolic pathways in tissues of animals, and metabolic transition through final products is of considerable ecological importance. Though the DDT metabolic processes have been well described by Kelvin, *et al*. (1969), additional detail for varying aquatic organisms are required. The intent of this communication is to demonstrate the peculiarities of accumulation and distribution of residues of DDT and its metabolites in organs and tissues of freshwater fish. Further, the factors characterizing the development of intoxication will be considered.

Experimental efforts directed toward three major areas: 1) a demonstration of the level of persistent pesticides in the aquatic ecosystems and the organisms under examination; 2) perform experiments *in vitro* to demonstrate the accumulation of residues of DDT and its metabolites in selected organs and tissues of fish, and to describe the developmental characteristics of the intoxification process in time; and 3) conduct studies in experimental basins to establish accumulation and transformation of persistent pesticides at different trophic levels. In these studies, the following fish species were used: bream (*Abramis brama*), pike perch (*Lucioperca lucioperca*), pike (*Esox esox*), perch (*Perca fluviatilis*), carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*), silver carp (*Hypophthalmychthys molitrix*). The food organisms tested included tubificids (*Tubifex tubifex*), and water fleas (*Daphnia magna*).

The residue level of DDT and its metabolites in water, silt, and tissues of fish was determined by the gas chromatography technique.

Systematic examination for DDT and its metabolites (DDE and DDD) in the water and sediments of the investigated water-bodies showed that this pesticide was not always found. Their concentration in water were found to be in the parts per trillion (ppt) and (ppb) parts per billion range. Sediment values were in the range of parts per billion (ppb) and parts per million (ppm). Since DDT solubility in water is expressed by a range of 1-5 ppb, the availability of DDT and its metabolites in freshwater ecosystems is not a function of physio-chemical transformations, but rather of biological transformation of this substance, and its accumulation in trophic levels on the basis of biological increases of 1 order of magnitude per trophic level. As a result, it is possible to find rather high concentrations accumulated in the second, third, and subsequent links of trophic chain. In both biologic tissues and in the abiotic environment, DDT alone is not isolated. Rather, the sum of its metabolites, DDD and DDE together with DDT proper is usually expressed as the sum of DDT (DDE + DDD + DDT).

In freshwater fish (pike perch, bream, pike, carp, perch, etc.) from the water-bodies investigated, the distributions of accumulated DDT and its metabolites in organs and tissues is rather clearly observed, although the content of DDT and its metabolites in tissues is comparatively low. The greatest accumulation of DDT residues is found in the inner fat and brain tissue of fish. Internal organs (liver, stomach and intestine) contain a considerable quantity of the metabolites (DDE and DDD), but comparatively little DDT. An even lesser amount of residual DDT is found in gonads and spawn, while the lowest levels of residues of this pesticide are found in muscular tissue (Komarovskiy, et al. 1975).

Thus, residual quantities of DDT and its metabolites are mainly accumulated in fatty and brain tissues. Having been taken into the fish, DDT undergoes substantial metabolic changes. This fact is indicated by predominance of the metabolites DDE and DDD in storage organs.

It should also be noted that the results did not demonstrate the presence of polychlorinated biphenyls in organs and tissues of fish from the study sites. However, corresponding analysis of fish specimens from the Black Sea and the Barents Sea were positive for the presence of PCB (chromatograms showed saw-tooth peaks, analogous to those of the Baltic fish that were convincingly shown by Swedish scientists to be associated with PCB's). Chromatograms of the freshwater fish associated with the present investigation showed only peaks typical for DDT and its metabolites.

The experimental research associated with this study provided the opportunity to confirm data on specific differences in accumulation and distribution of DDT residues in fish tissue, and to demonstrate differences conditioned by the functional role of tissues, and the metabolic rate of DDT during intoxication. Pesticide accumulation depends upon metabolic activity. For example, DDT accumulation is much greater in tissues of predatory fish, notable for their elevated level of metabolism. Total DDT content in the liver of fish from the experimental water-bodies was as follows: pike - 1.400 ppm, zander - 0.220 ppm, silver carp - 0.115 ppm, and carp - 0.047 ppm. Crucian carp, subjected to the effect of high concentrations (40 ppm) of this pesticide had DDT accumulation in intestine 0.850 ppm by the end of the exposure, while pike perch had 1.430 ppm.

Pesticides accumulation was conditioned by the functional role of tissues. It was the greatest in the tissues playing an important role in the detoxification of pesticides (liver), and those having a comparatively high content of lipid (liver, inner fat, and intestine). For example, total DDT uptake under experimental intoxication for pike perch was as follows: liver - 0.220 ppm, intestine - 3.175 ppm, inner fat - 5.635 ppm, muscles - 0.057 ppm.

The clinical picture of fish intoxication as a result of acute DDT exposure was characterized by a marked behavioral change. Intensive locomotor activity gave way to deceleration and a disturbance of coordinative movements, loss of balance, adynamia and death. Dissection of the fish revealed marked hemorrhaging of the brain and other vital organs (gills, liver, heart, kidneys, etc.), as well as necrotic changes, especially in the liver.

Chromatographic analysis showed comparatively rapid (within hours) accumulation of DDT and its metabolites (o,p' - DDE, o,p' - DDD, o,p' - DDT, p,p' - DDD and p,p' - DDT) in fish tissues. Estimation of the DDT residue content at different phases of intoxication enabled an understanding of the dynamics of this process during fish convulsions (Phase 1), and at adynamia, preceding death (Phase 2).

The quality of DDT and its metabolites increased in the tissues during the processes of the development of intoxication, within a few hours. Total DDT content in the muscles of silver carp increased from 0.103 ppm during the first phase to 0.501 ppm at the second phase of intoxication. In liver this increase was from 1.99 ppm during the first phase to 3.38 ppm during the second phase. Similarly, in the intestine the range was from 2.83 ppm at the first phase up to 0.79 ppm during the second phase.

Accumulation of DDT and its metabolites in fish was also accompanied by a phase change of a number of biochemical indices, the group B vitamins in particular. For example, vitamin B_1 content increased in carp liver by 131 percent when locomotor activity was increased (Phase 1), and decreased by 14 percent at the time of adynamia (Phase 2) when compared with control values. These data are indicative that vitamins are of considerable importance in the process of intoxication.

During the first phase of intoxication, the vitamin B_1 content, which is of considerable importance in metabolic processes, increases. During the second phase when metabolism processes are disturbed, the organism's vital resources are exhausted and the vitamin B_1 quantity is greatly reduced.

Changes in the levels of nicotine-amide enzymes in the fish tissues were also indicative of alterations in the oxidation-reduction processes. The total quantity of oxidized and reduced forms of nicotine-amide enzymes decreased in fish liver as a result of the action of lethal quantities of DDT, from 554 ppm in the control group to 307 ppm in test animals. Similarly, the ratio of oxidized and reduced forms also decreased in the liver tissue from 2.26 ppm in control fish to 0.96 ppm in test species. Since nicotine-amide enzymes are of great importance in the regulation of cellular respiration, the alterations observed were indicative of considerable metabolic disturbances in fish tissues under the influence of DDT.

Coupled with these observations was an extensive formation of metabolites of DDT in organs and tissues rich in lipids. The formation of p,p' - DDE; o,p' - DDT; p,p' - DDD; p,p' - DDT metabolites in intestine and inner fat were of analogous character. DDT accumulation in fatty tissue during the first phase of intoxication is accompanied by the formation of the metabolite n,n' - DDD, while levels of o,p' - DDT and p,p' - DDE increase. During the second phase this ratio changed to domination by p,p' - DDD and o,p' - DDT. In the intestine, p,p' - DDT, and o,p' - DDT predominated during the first phase, and by the second phase p,p' - DDD was dominant. In the muscles of silver carp during the first phase of intoxication, p,p' - DDT content was the greatest, while o,p' - DDT and p,p' - DDD were pronounced in the second phase.

The liver, unlike other organs, was notable for greater stability in content of DDT metabolites. This was conditioned by rapid transformation of DDT in this organ. During the second phase of intoxication, o,p' - DDT, p,p' - DDT, and p,p' - DDD were predominant.

Thus, the accumulation of DDT and its metabolites in organs and tissues of fish is conditioned by their specific peculiarities, functional purpose, and time of development of intoxication.

With the intent of studying accumulation of persistent pesticides, the level of transformation in aquatic organisms, and their distribution and transmission in trophic chains, experiments in aerated aquaria and pools were carried out. In the process of studying the transformation of DDT and its metabolites in the food chain, forage organism (Tubifex tubifex and Daphnia magna), consumer fish (Cyprinus carpio), and predatory fish (Perca fluviatilis and Esox lucius) were modeled.

Food organisms poisoned by chemically pure p,p' - DDT (1.1 to 3 ppm) were fed to yearling carp, which in turn were eaten by predatory fish. Control fishes were given food without DDT. During the experiments, DDT accumulation and metabolism at selected levels of the trophic chain were controlled, and the complex of morphlogical and functional indices characterizing the development of intoxication were studied (Braginskiy, et al. 1976).

Investigations have shown that the DDT residue from water was taken into the tissues of the Daphnia and tubificids in a very short time period, practically within the first day. When these organisms were fed to fish, considerable concentrations of DDT residues were found in organs and tissues, especially in fatty layers and in brain tissues, as early as the first 3 days, with a constant increase throughout the experiments. In the forage species, (Daphnia and the tubificids), DDT metabolizes primarily to DDD, while DDE is formed very slowly. In carp, the general accumulation of pesticides with high specific weights of the DDE metabolite greatly increase. An analogous picture is characteristic of perch and pike. When these species are fed for an extended time with food containing DDT, the accumulation of this substance in their lipid containing tissues increases, with a prevalence of the metabolites DDE and DDD.

Tubificids metabolize DDT only to DDD; Daphnia to DDD and DDE, carp to DDD and DDE, and perch and pike to DDD and DDE, but with different percentage ratio.

Experimental research has shown that in parallel with fatty tissue, DDT accumulates extensively in fish brain tissue, reaching critical values (Braginskiy, et al. 1979). It was found that using poisoned natural food, the developing of intoxication in fish was, in fact, connected with accumulation of DDT and its metabolites. It was stated that the fish died from toxicosis at critical levels of DDT accumulation in the brain (3 ppm and greater). These findings correspond to the results obtained during the investigation of analogous phenomena in warm-blooded animals (Dale, et al. 1963).

The modeling experiments show that DDT accumulates in trophic chain quickly and effectively. Accumulation of DDT and its metabolites in fish organs of vital importance was observed. These findings were distinctly manifested in the fish brain tissue.

Toxicological symptoms appear in parallel with increasing levels of DDT in target organs, especially in the brain. Clinical and pathological-anatomical intoxication may be reproduced by experimental modeling rather quickly and synonymously, and the fish behavior and clinical symptoms are similar to those of acute intoxication.

When DDT and its metabolites (DDE and DDD) accumulate up to 3 ppm in the brain tissue of fish (perch, pike), the fish die with obvious symptoms of cumulative toxicosis. It should be noted that mammals and birds present an analogous picture, i.e., convulsive phenomena as DDT accumulation approaches the lethal level, and death at definite accumulation levels (Hayden 1960; Dale, et al 1963; Ludwig and Ludwig 1969).

Thus, these investigations enabled the development of principles of the actions of DDT and its metabolites. Their distribution in organs and tissues of freshwater fish, the development of a model of cumulative toxicosis in fish under experimental conditions, and an understanding of the basis of accumulation of DDT, along with its metabolism, depending upon the functional role of the tissue and species of aquatic organism.

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SECTION 10

SOME FACTORS AFFECTING THE TOXICITY OF AMMONIA TO FISHES

Robert V. Thurston[1]

INTRODUCTION

Ammonia can be a serious toxicant to fishes and other aquatic life. It can enter natural water systems from several sources, including industrial wastes, sewage effluents, coal gasification and liquefaction conversion process plants, and agricultural discharges including feedlot runoff. It is also a metabolic waste product of fishes, and as such presents a major problem in fish culture.

In aqueous solutions, ammonia assumes two chemical species, illustrated by the following equation.

$$NH_{3(g)} + nH_2O_{(\ell)} \rightleftarrows NH_3 \cdot nH_2O_{(aq)} \rightleftarrows NH_4^+ + OH^- + (n-1)H_2O_{(\ell)}$$

These species are the gaseous or un-ionized form (NH_3), bound to at least three water molecules, and the ionized form (NH_4^+). In this presentation, the term NH_3 will refer to un-ionized ammonia, NH_4^+ will refer to ionized ammonia, and total ammonia will refer to the sum of these. The aqueous ammonia equilibrium is strongly dependent upon the pH of the solution, and to a lesser extent upon temperature and ionic strength. As the pH increases, increasing the hydroxide ion concentration, the equilibrium shift of ammonia is toward the un-ionized (NH_3) species. Within the pH range acceptable to most freshwater fishes, an increase of one pH unit will increase the NH_3 concentration approximately tenfold (Thurston *et al.* 1974). Temperature increase also favors the NH_3 species, but to a lesser extent; ionic strength increase, at low concentrations, favors the NH_4^+ species (ibid).

Early reported research on the toxic effect of ammonia (Chipman 1934; Wuhrmann *et al.* 1947; Wuhrmann and Woker 1948) implicated NH_3 as being the toxic form of ammonia, and NH_4^+ was considered non-toxic or appreciably less toxic. Because of the recognized toxicity of NH_3, and the belief that NH_4^+ is not significantly toxic, most toxicity values reported in the literature are as NH_3. Sometimes total ammonia values have also been reported, but too frequently pH, temperature, and other water quality parameters have been omitted, making it difficult to reconstruct reported test conditions.

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Much of the literature on ammonia toxicity to fishes has recently been reviewed in the EPA "Red Book" (U.S. EPA 1977) and the American Fisheries Society "Red Book Review" (Thurston et al. 1979). Reported acute toxicity values in tests from 1 to 4 days duration on salmonids range from 0.25 to 0.85 mg/liter NH_3; values for comparable tests on non-salmonids range between 0.4 and 4 mg/liter NH_3.

Published reports on chronic toxicity of ammonia do not include any life-cycle mortality data, but effects of ammonia on both warm- and cold-water fishes at sublethal concentrations of ammonia for periods of time ranging from 1 week to 3 months have been reported by several researchers. Within the concentration range of 0.06 to 0.4 mg/liter NH_3, these reported effects include swelling and diminishing of number of red blood cells, irreversible blood damage, inflammation and degeneration of gills and other tissues, and lessening of resistance to disease (Reichenbach-Klinke 1967; Flis 1968; Smart 1976). Within the range 0.05 to 0.15 mg/liter NH_3, reduced food uptake and assimilation and growth inhibition have been reported (Ministry of Technology 1972; Robinette 1976; Schulze-Wiehenbrauck 1976; Burkhalter and Kaya 1977). In a test of 6 months duration on rainbow trout (Salmo gairdneri) it has been reported that concentrations as low as 0.01 mg/liter NH_3 caused not only reduced growth rates, but pathological changes to gills and livers (Smith and Piper 1975). Ball (1967) indicated that although it may appear that different species of fishes exhibit dissimilar susceptibilities to ammonia toxicity under acute exposure conditions, such is not the case under long-term exposures. He theorized that trout and carp, given time to react, may be equally susceptible to ammonia, and that although acute responses are different, the ultimate response by both fishes to a given concentration of ammonia may be the same.

In summary, reported acute toxicity ammonia values for a variety of species of fishes range between 0.25 and 4 mg/liter NH_3, and other manifestations of the effects of ammonia have been reported at concentrations as low as 0.01 mg/liter NH_3. There is some evidence that differences in ammonia tolerance among fish species may be less under chronic conditions than under acute conditions. Based on the published literature, the European Inland Fisheries Advisory Commission (EIFAC 1970) has recommended a criterion of 0.025 mg/liter NH_3 as being the maximum which can be tolerated by fishes for an extended period of time, and the United States Environmental Protection Agency (1977) has published a criterion of 0.02 mg/liter NH_3, just slightly more restrictive than that recommended by EIFAC.

Very possibly these criteria are "safe" for most water bodies which support aquatic life, but some questions remain unanswered as to whether they are reasonable for all waters at all times under all conditions. Tabata (1962) has attributed some toxicity to NH_4^+, concluding that it may be 1/50th as toxic as NH_3 to Daphnia pulex. Robinson-Wilson and Seim (1975), testing coho salmon (Oncorhynchus kisutch), have demonstrated correlation between pH and the acute toxicity of ammonia expressed as NH_3. More recently Armstrong et al. (1978), in tests on larvae of the prawn Macrobra-

chium rosenbergii, concluded that NH_4^+ is toxic. The work of these researchers raises questions about an ammonia criterion based solely on NH_3. In addition, it is also known that prior acclimation, temperature, and dissolved oxygen may also affect the toxicity of ammonia to fishes. Considering the large number of industrial and agricultural discharges which contain ammonia, and the tremendous expenditure of energy and resultant cost to treat these discharges for ammonia reduction to meet statutory requirements, it is reasonable to ask whether a single water quality standard for ammonia can be justified. Certainly some of the factors that increase or decrease the toxicity of ammonia should be considered further.

EFFECT OF ACCLIMATION

The question of whether fishes can acquire an increased tolerance to ammonia by acclimation to low ammonia concentrations is an important one. In certain real-world environmental situations, such as a stream receiving effluent from a sewage treatment plant, fishes may be subjected to high ammonia concentrations for short and/or intermittent periods of time. If a fish had an increased ammonia tolerance, developed due to acclimation or conditioning to low ammonia levels, it would perhaps be able to survive what might otherwise be acutely lethal ammonia concentrations.

There is some information in the literature reporting that the effect of previous exposure of fishes to low ammonia concentrations reduces or does not affect their tolerance to lethal ammonia levels. Steinmann (1928) reported that the minnow *Alburnus bipunctatus* was more susceptible to ammonium hydroxide if previously exposed. Observations by McCay and Vars (1931) indicated that bullheads (*Ameiurus nebulosus*) subjected to several successive exposures to ammonia, alternated with recovery in fresh water, acquired no immunity from the earlier exposures to the later ones. Fromm (1970) acclimated goldfish (*Carassius carassius*) to low (0.5 mg/liter) or high (5.0 or 25.0 mg/liter) ambient NH_3 for periods of 20 to 56 days and found that urea excretion rate in subsequent 24-hour exposures to concentrations ranging from 0.08 to 2.37 mg/liter was independent of the previous acclimation concentration or duration.

There is a larger body of information, however, which indicates that prior exposure of fishes to low concentrations of ammonia increases their resistance to lethal concentrations. Vámos (1963) conducted an experiment in which carp (species not specified) were exposed to 0.67 and 0.52 mg/liter NH_3 for 75 minutes, revived in fresh water for 12 hours, and then subjected to ammonia at a concentration of 0.7 mg/liter NH_3. Control fish, exposed only to the latter ammonia concentration, developed ammonia-poisoning symptoms within 20 minutes, but the previously exposed fish did not exhibit these symptoms until 60-85 minutes. Mălăcea (1968) subjected carp (*Rhodeus sericeus amarus* Bloch) for 4 days, and minnows (*Phoxinus phoxinus* L.) for 3 days to "acclimation" solutions of ammonium sulfate (0.26 mg/liter NH_3). The "adapted" carp and "unadapted" control group were then exposed to lethal concentrations of ammonium sulfate (5.1 mg/liter NH_3). The mean survival time of the adapted carp was 88 minutes and that of the unadapted carp was 78 minutes. The minnows were subjected to lethal toxic concentrations of

2.4 mg/liter NH_3 in ammonium sulfate solution. Mean survival time of adapted minnows was 65 minutes, and of the unadapted control group was 45 minutes.

Fromm (1970) has measured urea excretion rates of rainbow trout initially subjected to either 5 or 0.5 mg/liter NH_3, and then subjected to 3 mg/liter NH_3. The trout previously exposed to 5 mg/liter NH_3 excreted slightly less urea than those previously exposed to the lower concentration. Lloyd and Orr (1969) measured urine flow rates of rainbow trout exposed for 24 hours to 0.27 mg/liter NH_3, and then exposed for another 15 hours to 0.53 mg/liter NH_3. Pretest urine flow rates of 2.8 ml/kg/hr increased first to 6.4 and then to 8.0. One fish died during the lower ammonia level exposure and none during the higher exposure. A control batch of fish with a pretest urine flow rate of 0.75 ml/kg/hr was subjected directly to the higher (0.53 mg/liter NH_3) ammonia concentration. The urine flow rate jumped to 11 ml/kg/hr, and all fish died within 3 hours.

In a second experiment by Lloyd and Orr (1969), rainbow trout were subjected to 0.32 mg/liter NH_3 for successive 22-hour time periods, separated by a 24-hour non-exposure period. Although urine flow rates were higher during exposure periods than during pre-exposure, they were less during the second exposure period than during the first. This suggests that some acclimation was developed and subsequently retained, at least for a 1-day rest period. A third experiment indicated that this acclimation was not retained during a 3-day rest period between two similar ammonia exposures.

Schulze-Wiehenbrauck (1976) conducted a study on the effect of sublethal ammonia exposures on young rainbow trout growth, food consumption, and food conversion. In one experiment, trout were acclimated for 3 weeks at 0.007 (control), 0.131, and 0.167 mg/liter NH_3; the fish from these three tanks were then subjected to concentrations of approximately 0.45 mg/liter NH_3 for 8.5 hr. Fish from the two ammonia acclimation concentrations had 100 percent survival, whereas only 50 percent of the control group survived the test period. In the second experiment, the acclimation concentrations were 0.004 (control) and 0.16 mg/liter NH_3; these fish were placed in NH_3 concentrations of approximately 0.5 mg/liter for 10 hours. There was 100 percent survival of the ammonia acclimated fish, and 85 percent survival of the control fish. The results of these experiments thus showed an increase in resistance of rainbow trout to acutely toxic concentrations of ammonia after prior exposure to sublethal ammonia concentrations.

At Fisheries Bioassay Laboratory we have conducted experiments to investigate the effect of acclimation of rainbow trout to sublethal ammonia concentrations on the fish's response to acutely lethal ammonia concentrations. Seven 96-hour flow-through bioassays (using NH_4Cl) were conducted, six of these on fish that had been acclimated for 29 days to concentrations ranging from 0.018 to 0.078 mg/liter NH_3, and the seventh on a control group acclimated at 0.001 mg/liter NH_3. For each bioassay there were 5 test tanks and 1 control tank containing 10 fish each; mean fish sizes for the tests were 12 to 15 g. Additional details of these tests and data treatment will be reported elsewhere (Thurston and Russo, in preparation).

Figure 1 shows the toxicity curves for these tests (LC50 in mg/liter NH_3 vs. time). There was a statistically significant correlation between the NH_3 concentration at which the fish were acclimated and their subsequent resistance to acutely toxic NH_3 concentrations. The higher the NH_3 concentration at which the fish were acclimated, the more tolerant the fish were to acutely lethal levels during the 96-hour test period. The shapes of the curves also show that there is a general trend for fish acclimated at higher ammonia concentrations to take longer to arrive at an eventual asymptotic LC50 value.

We also performed some experiments to determine whether the length of time of acclimation to low ammonia concentrations affected the fish's response in subsequent exposure to lethal NH_3 levels. Duration of acclimation to ammonia in these experiments ranged from 29 to 154 days; the subsequent lethal tests were all 96-hour bioassays as described above. Results showed that there was a significant relationship between 96-hour LC50 and length of time of prior acclimation; the longer the acclimation period, the more tolerant the fish were to high ammonia levels. Our calculations took into consideration the fact that fish weight also increased as acclimation duration increased. We also investigated whether there was an effect on fish's tolerance to ammonia if they were placed in fresh (ammonia-free) water for periods of 2, 14, and 28 days after acclimation and before exposure to lethal concentrations. From limited data, our experiments indicated that fish rapidly (less than 2 days) started to lose the tolerance to ammonia built up by acclimation once they were placed in ammonia-free water.

In summary, there is reasonable evidence that fishes with a history of prior acclimation to some sublethal concentration of ammonia are better able to withstand an acutely lethal concentration, at least for some period of hours and possibly days. The concentration limits for both acclimation and subsequent acute response need definition and explanation.

EFFECT OF TEMPERATURE

There is limited information in the literature on the effects of temperature on ammonia toxicity to fishes. Generally, the toxicity of total ammonia decreases with lower temperatures, attributable mainly to a decrease in the concentration of NH_3. Woker (1949), testing chub (*Squalius cephalus*) within the range of 10-25 C, concluded that water temperature had practically no effect on the manifestation time of toxic symptoms resulting from ammonia. On the other hand, Colt and Tchobanoglous (1976) observed that the tolerance of channel catfish (*Ictalurus punctatus*) to ammonia increased as the experimental temperatures were increased up to the fish's reported optimum temperature for growth (29-30 C). It is reasonable to expect that at temperature conditions which are marginal for any given fish species, the species will not be able to function optimally to resist toxic effects of ammonia.

We have conducted eight 96-hr ammonia bioassays on 2- to 12-g rainbow trout at elevated temperatures within the range 12-19 C. Test conditions were similar to those employed in the acclimation experiments reported

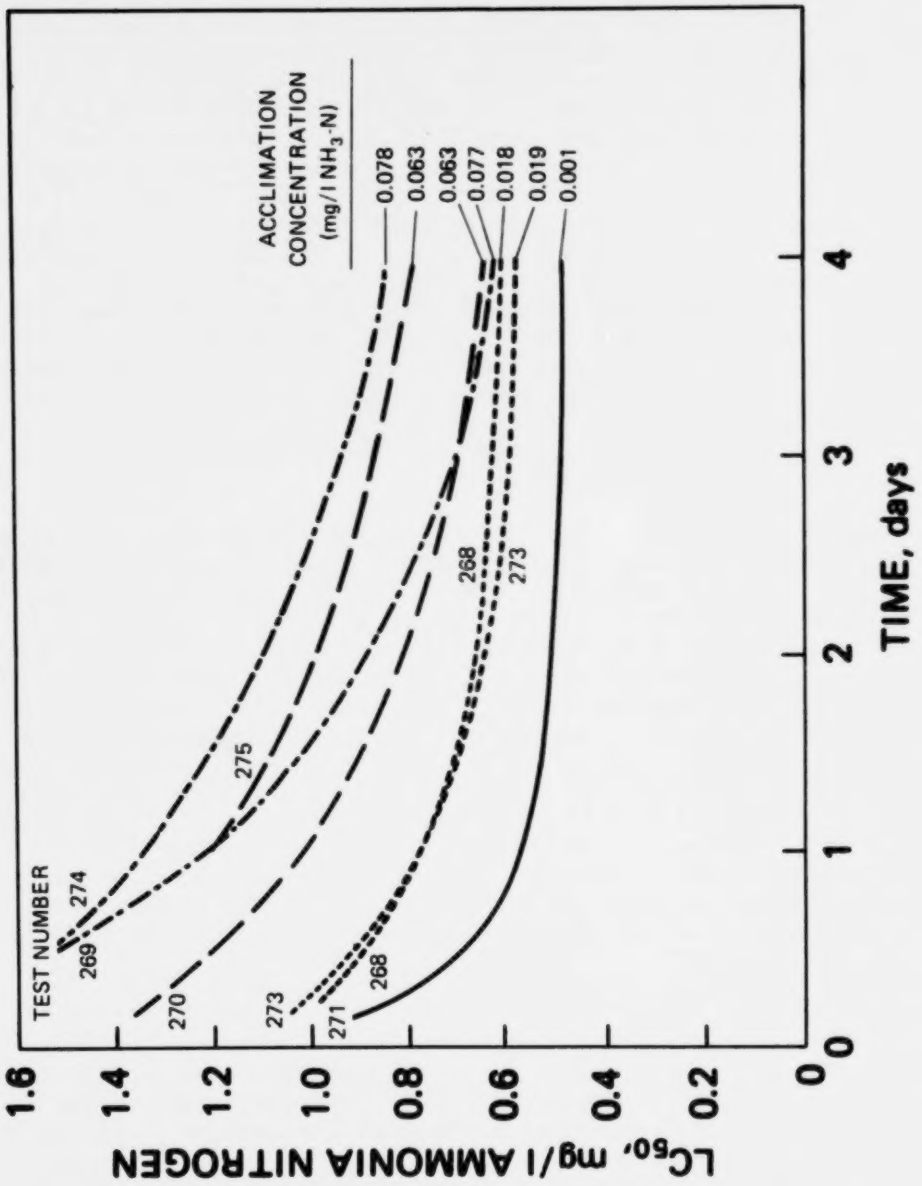

Figure 1. Effect of prior ammonia acclimation on the acute toxicity of ammonia to rainbow trout.

above. Fish were acclimated to test temperature for 1.5 to 2 days prior to introduction of ammonia toxicant. Ninety-six hour LC50 values ranged between 0.6-1.2 mg/liter NH_3, but there was no correlation between ammonia toxicity and temperature. Statistical treatment showed that size was not a factor. We also conducted nine similar tests on 1-g cutthroat trout (*S. clarki*), within the range 13-19 C. Ninety-six hour LC50 values ranged between 1.0-1.5 mg/liter NH_3, but again there was no temperature/ammonia toxicity relationship. In 15 tests on fathead minnows (*Pimephales promelas*), however, within the range 13-22 C, we did find a definite correlation between temperature and susceptibility to ammonia toxicity. The toxicity curves for these tests are shown in Figure 2. As temperature decreased, toxicity increased. A plot of 96-hour LC50 values (mg/liter NH_3) vs. temperature, and a statistically computed correlation curve are illustrated in Figure 3. It should be noted that in the case of the two trout species tested, the temperature range studied was above their normal environmental temperature; in the case of the fathead minnows, the range tested reached several degrees below that for their optimum growth. We have not tested fathead minnows at temperatures above, nor have we tested trout below, their optimum growth temperature ranges.

Our results for trouts agree with those reported by other researchers within the temperature range 10-20 C (Herbert 1962; Lloyd and Orr 1969). The British Ministry of Technology (1968), however, has reported that the toxicity of ammonia to both adult and juvenile rainbow trout was much greater at 5 C than at 18 C. Based on our analysis of their data as reported, their case for juvenile trout appears stronger than that for adults. The European Inland Fisheries Advisory Commission (1970) has cautioned that acceptable concentrations of ammonia may be less at temperatures below 5 C. Although this temperature value may be arbitrary, we conclude that there is some merit to the argument that a drop in temperature below some optimum range for a given species of fish may increase its susceptibility to ammonia toxicity. It is important that this relationship be further studied. The available evidence that temperature, independent of its role in the aqueous ammonia equilibrium, affects the toxicity of ammonia to fishes argues for further consideration of the temperature/ammonia toxicity relationship.

EFFECT OF DISSOLVED OXYGEN

The discharge of ammonia is frequently associated with a reduction in oxygen levels in the receiving water. This is brought about by any of several causes, including the oxygen demand of the ammonia itself as it is converted by natural microbial oxidation to nitrite and nitrate; the chemical and biological oxygen demand of other chemicals which may be, and frequently are, discharged along with ammonia; and the reduction in oxygen-carrying capacity of the receiving water if the discharge causes a rise in its temperature. If the receiving water body is rich in nutrients and highly productive, as is frequently the case downstream from a sewage treatment plant, there is the effect of diurnal and seasonal fluctuations in dissolved oxygen caused by plant growth.

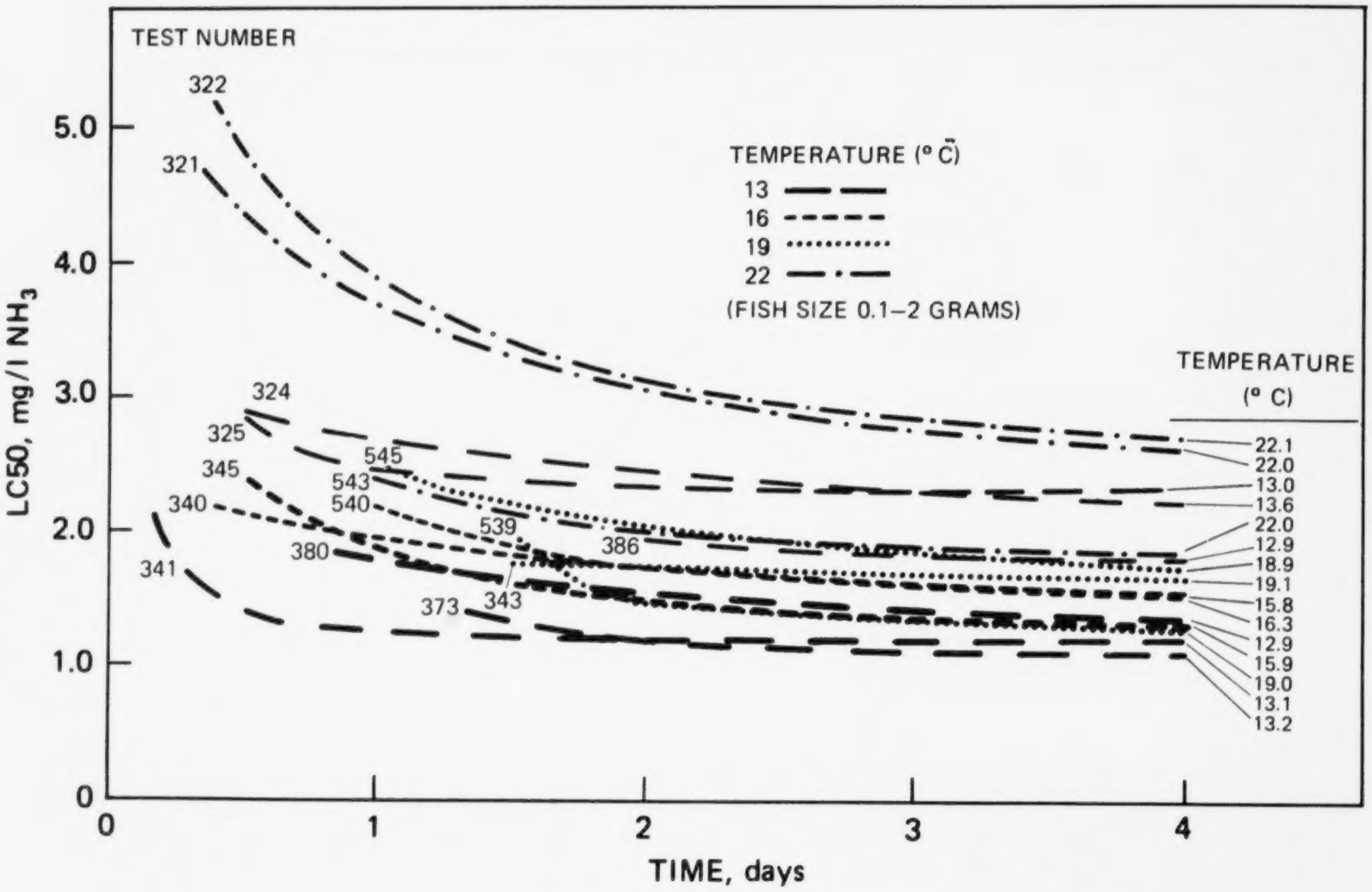

Figure 2. Effect of reduced temperature on the acute toxicity of ammonia to fathead minnows.

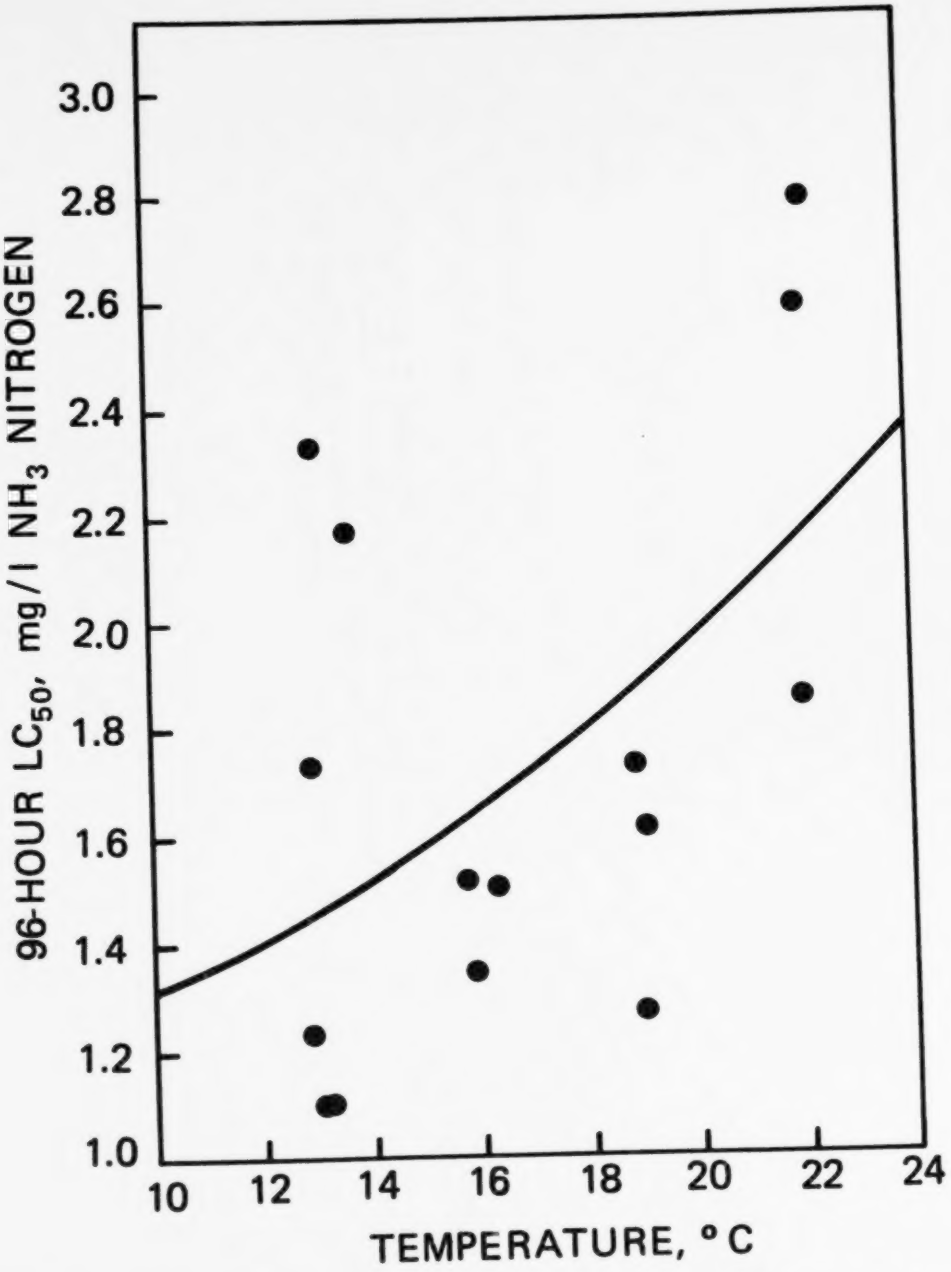

Figure 3. Actue toxicity of ammonia vs. temperature for fathead minnows. [LC50 = 1.086 + 0.002203 $(\text{temperature})^2$].

Several researchers, working with a variety of warm-water fishes, have reported that the acute response to ammonia was not affected when dissolved oxygen levels dropped from saturation to approximately one-half or one-third saturation, but below that resistance decreased (Wuhrmann 1952: Wuhrmann and Woker 1953; Merkens and Downing 1957; Danecker 1964; Vámos and Tasnádi 1967). Reports on rainbow trout generally agree that this species is more sensitive than warm-water fishes to the combined effects of low dissolved oxygen and ammonia, and that any reduction in dissolved oxygen or any reduction below two-thirds saturation will decrease rainbow trout tolerance to ammonia (Allan 1955; Downing and Merkens 1955; Merkens and Downing 1957; Danecker 1964). One of the findings reported by Downing and Merkens (1955), who tested young rainbow trout in experiments lasting up to 17 hours, was that a decrease in dissolved oxygen from 8.5 to 1.5 mg/liter shortened the periods of survival at all ammonia concentrations tested; this decrease was proportionally greatest at the lowest concentrations of ammonia. In longer tests, lasting up to 13 days, these same researchers reported similar results (Merkens and Downing 1957).

To explain the accelerated action of ammonia toxicity under reduced oxygen conditions, Lloyd (1961) presented the argument that a given toxic effect is produced by a specified concentration of toxicant passing across the fish gill surface at a rate governed by the fish gill movement. At reduced oxygen concentrations the rate of movement increases, resulting in an increased rate of gill exposure to the toxicant. He hypothesized that a reduction in CO_2 excretion at the gill surface, resulting from reduced O_2 intake, will raise the pH at the gill surface. Such an increase in pH will favor the more toxic ammonia species (NH_3) resulting in an even more accelerated toxic effect of ammonia than might be expected solely by an increased rate of gill movement. However, CO_2 loss at the gill surface is also connected with the fish's ammonia excretion mechanism, and recent research on the possible toxicity of NH_4^+ suggests that a complete explanation may be more complex.

To examine the effect of dissolved oxygen on ammonia toxicity we conducted two series of 96-hour flow-through bioassays, one of these (15 bioassays) on rainbow trout, and the other (10 bioassays) on fathead minnows. Test conditions were similar to those described earlier, and test fish were acclimated to the test oxygen level for at least 2 days prior to introduction of ammonia toxicant. The rainbow trout for all tests were from the same stock, and the stock fish grew in size over the several weeks that the tests were conducted so the average test fish size gradually increased from 2 to 10 g. The tests were not run in any particular sequence of dissolved oxygen level, however, and subsequent statistical treatment showed that there was no correlation between test result and fish size. Figure 4 shows a plot of the 96-hour LC50 value (mg/liter NH_3) for each test vs. the dissolved oxygen level at which the test was conducted. The correlation for rainbow trout between LC50 and dissolved oxygen was striking (correlation coefficient 0.9346, P = 0.00001); the lower the dissolved oxygen concentration, the greater the toxicity of ammonia. Although a regression line for the fathead minnow tests was obtained, the slope of this line is not statistically different from zero (P = 0.365). We conclude that there is most de-

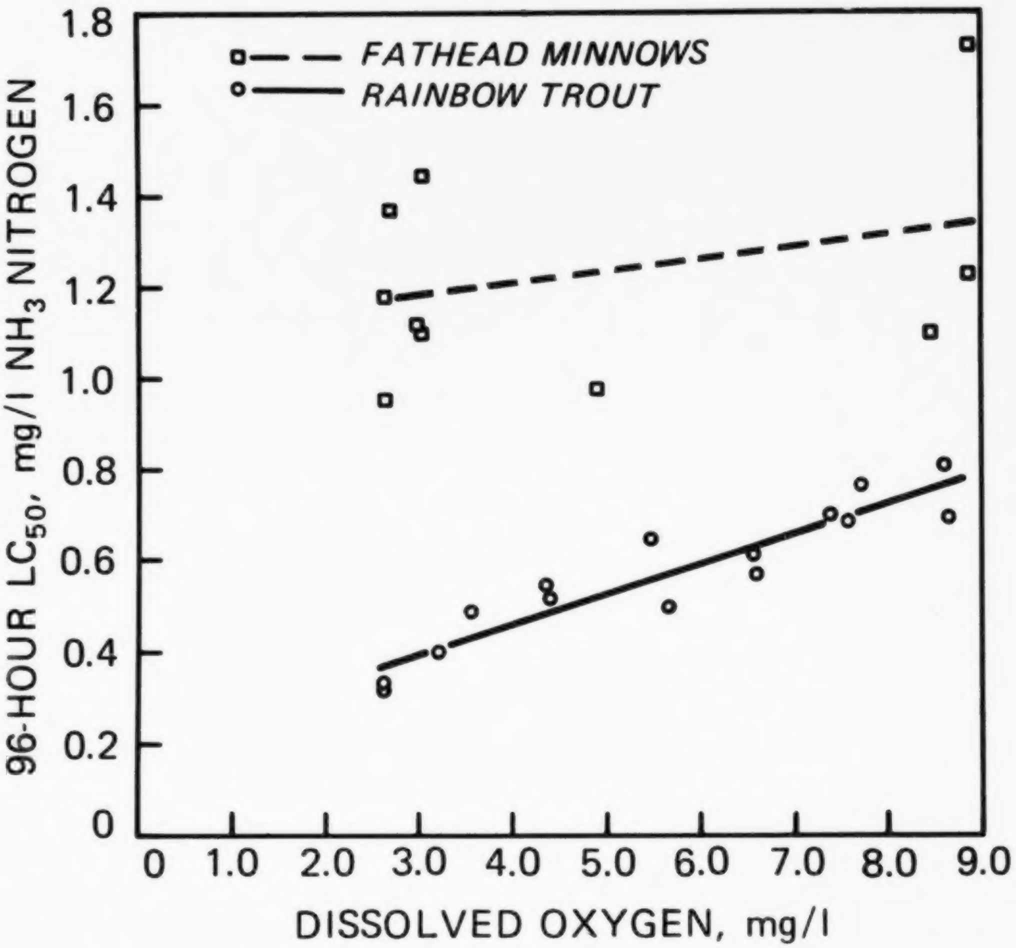

Figure 4. Effect of dissolved oxygen on the acute toxicity of ammonia to fathead minnows and rainbow trout.

finitely a correlation for the rainbow trout tests, but we cannot draw the same conclusion for the fathead minnow tests.

In an attempt to study the reduced dissolved oxygen effect on ammonia toxicity in relation to time, we analyzed our data for the rainbow trout tests, comparing the dissolved oxygen vs. LC50 correlations for the tests at 12, 24, 48, 72, and 96 hours. This showed a very clear and statistically defensible trend (Figure 5); the shorter the time period, the more pronounced the correlation. This trend suggests at least two possibilities: either individual fish which require higher oxygen concentrations succumb early in the tests, and/or those fish which do survive become increasingly acclimated to the ammonia and oxygen test conditions as time progresses.

The EPA Red Book (U.S. EPA 1977) has recommended a minimum concentration of 5.0 mg/liter dissolved oxygen to maintain good freshwater fish populations. At that dissolved oxygen concentration the regression line for the rainbow trout tests reported above indicates a 96-hour LC50 of 0.5 mg/liter NH_3 (Figure 4). At dissolved oxygen concentrations of 8.0 mg/liter and above, more common to natural cold-water fish habitats, the test results regression line indicates 96-hour LC50's in excess of 0.7 mg/liter NH_3. For this particular stock of test fish, tested under the given bioassay conditions, there was a 30 percent decrease in the medium lethal concentration of ammonia when the dissolved oxygen concentration dropped from 8 to 5 mg/liter. If this ammonia LC50/dissolved oxygen correlation bears up under further testing using this and other species, the need for reconsideration of both ammonia and dissolved oxygen criteria is clear.

EFFECT OF pH

A premise of both the EIFAC (1970) and the U.S. EPA (1977) criteria for ammonia is that NH_4^+ is not appreciably toxic to aquatic life. The empirical basis for this was mentioned earlier, and has been explained by the ability of NH_3 to diffuse across the gill membrane whereas NH_4^+ requires active transport. The research by Tabata (1962), Robinson-Wilson and Seim (1975) and Armstrong *et al*. (1978), however, raises questions about the criteria premise.

We have conducted two series of bioassays to investigate the toxicity of ammonia under different pH conditions. The fishes tested were rainbow trout and fathead minnows, and the pH range was 6.5 to 9.0. We chose this pH range because its limits are those recommended by the U.S. EPA (1977) as being the limits acceptable to freshwater fishes. We treated the data from each test by the trimmed Spearman-Karber method (Hamilton *et al*. 1977) to determine both the total ammonia and the un-ionized ammonia 96-hour LC50 values. Again, for each bioassay there were five test tanks at different ammonia concentrations and one control tank; each tank contained 10 test fish. The pH of the water in all tanks for any one test was uniform; this was achieved by adjusting the normal pH (7.8) of the test water either up by means of a metered sodium hydroxide solution, or down using a solution of hydrochloric acid. During any given test, the ammonia concentration, pH, and temperature in each test tank were monitored between 5 and 8 times, and

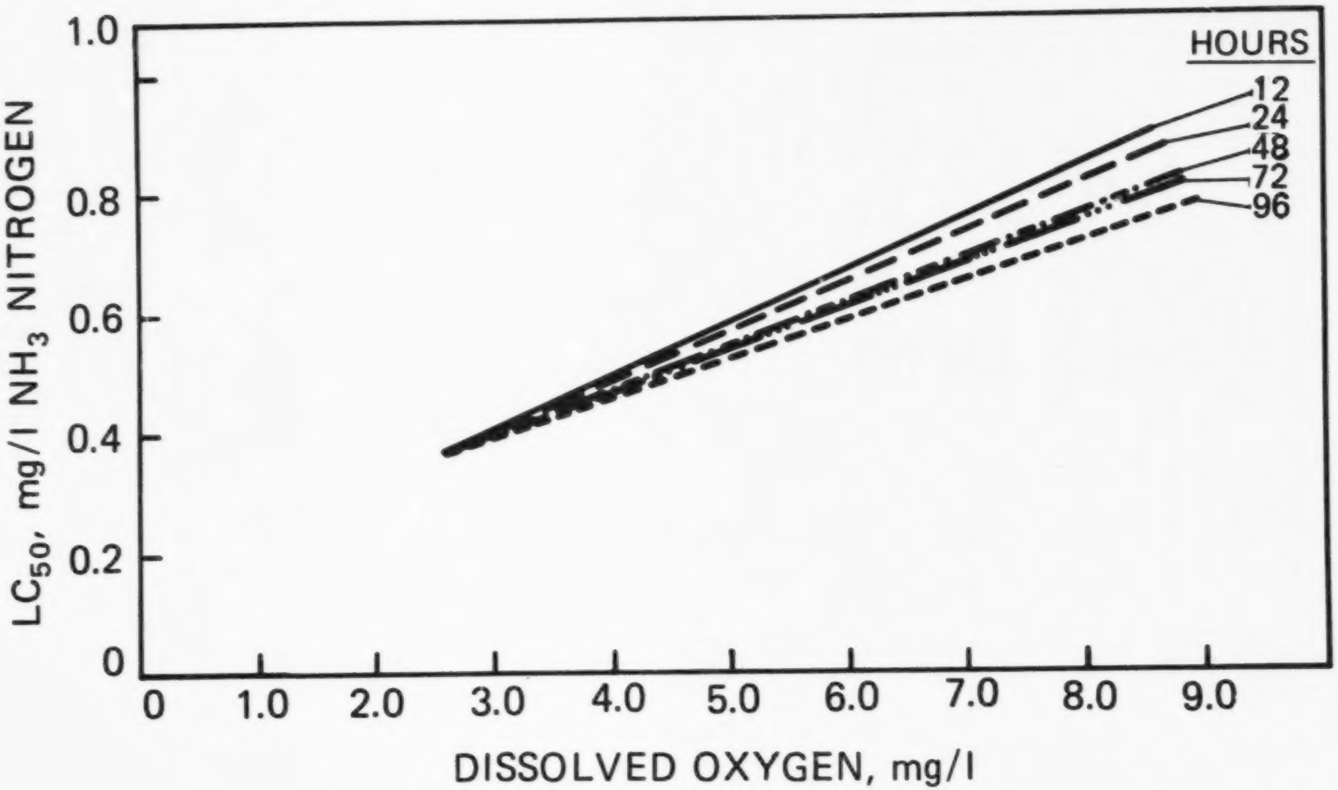

Figure 5. Effect of dissolved oxygen on the acute toxicity of ammonia to rainbow trout: LC50 vs. D.O. at 5 time intervals.

minute adjustments in pH were made as appropriate. Mixing of the test water and additives was virtually instantaneous, ensuring uniform water chemistry conditions throughout any one tank. This was confirmed by repeated sampling studies.

The average size of rainbow trout was 9-11 g, all fish from the same stock, and that for the fathead minnows was 1.8-2.0 g, again from a single stock. Tests were conducted on successive weeks, three at a time: one acid, one base, and one at the normal pH of the test water. The normal pH test was repeated each time the acid and base tests were conducted; comparable results from the normal pH tests verified that test conditions from week to week were comparable, and that the test fish stock had not changed appreciably over time.

The results of the tests on rainbow trout are illustrated in Figure 6. Ninety-six hour LC50 values and their confidence limits in terms of both total ammonia-nitrogen and un-ionized ammonia-nitrogen are plotted for each pH test. A log scale for the LC50 values has been used so that visual comparison of total ammonia and NH_3 values can easily be made. The excellent reproducibility of the tests run at normal test water pH is apparent. If the un-ionized form of ammonia (NH_3) were solely responsible for the toxic action on the test fish, then one would expect that the LC50 values, in terms of NH_3, would be reasonably constant for all tests regardless of the solution pH and total ammonia present. This did not turn out to be the case. Figure 7 illustrates the results of the tests on fathead minnows. The LC50 values, in terms of both total ammonia-nitrogen and un-ionized ammonia-nitrogen, are higher than those for rainbow trout because the fathead minnow is a more ammonia-tolerant fish, but the LC50 vs. pH trend is the same.

Our findings provide support for the conclusions of Tabata (1962) and Armstrong et al. (1978), and are in conflict with the more widely accepted notion that the toxicity of NH_3 is independent of pH. The LC50 values in terms of NH_3 for our 96-hour acute toxicity tests on rainbow trout are strikingly similar to those reported by Robinson-Wilson and Seim (1975) for coho salmon within the pH range 7.0 to 8.5. These authors explain the correlation of solution pH with NH_3 LC50 values to be related to changes in the CO_2 concentration, hence pH, at the surface of the fish gill tissue. Our conclusion at this time is that the NH_4^+ ion exerts a heretofore not fully recognized toxic effect on fishes, and/or that the toxicity of NH_3 increases as the H^+ ion concentration increases.

Regardless of the explanation for it, the correlation between LC50 in terms of NH_3 and pH has been demonstrated, and the rationale for water quality criteria for ammonia needs to address this.

CONCLUSION

I have discussed briefly just four factors affecting the toxicity of ammonia. I have used these as examples of how the many chemical and physical parameters involved in aqueous systems are interrelated in affecting the

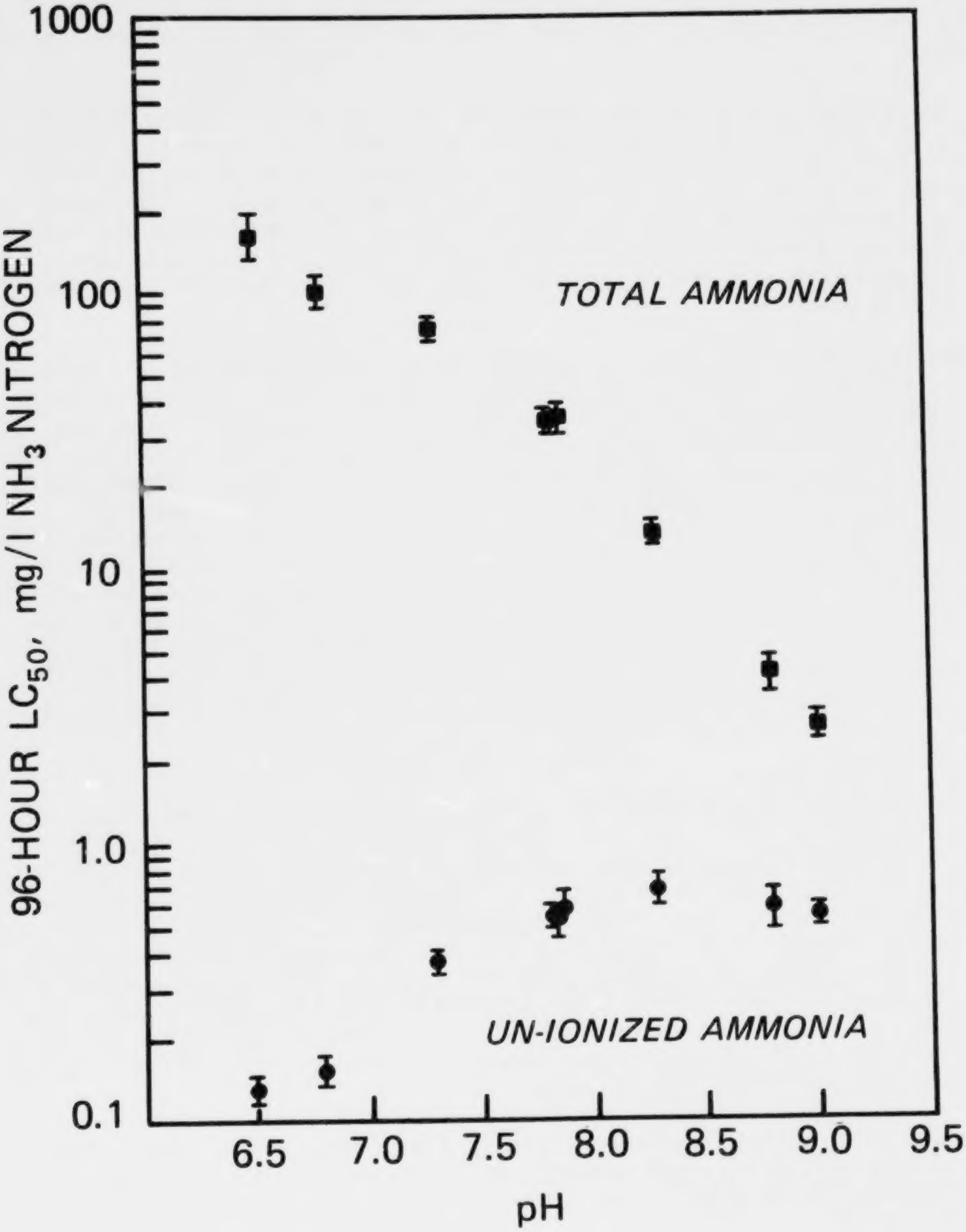

Figure 6. Acute toxicity of ammonia to rainbow trout: 96-hour LC50 vs. pH.

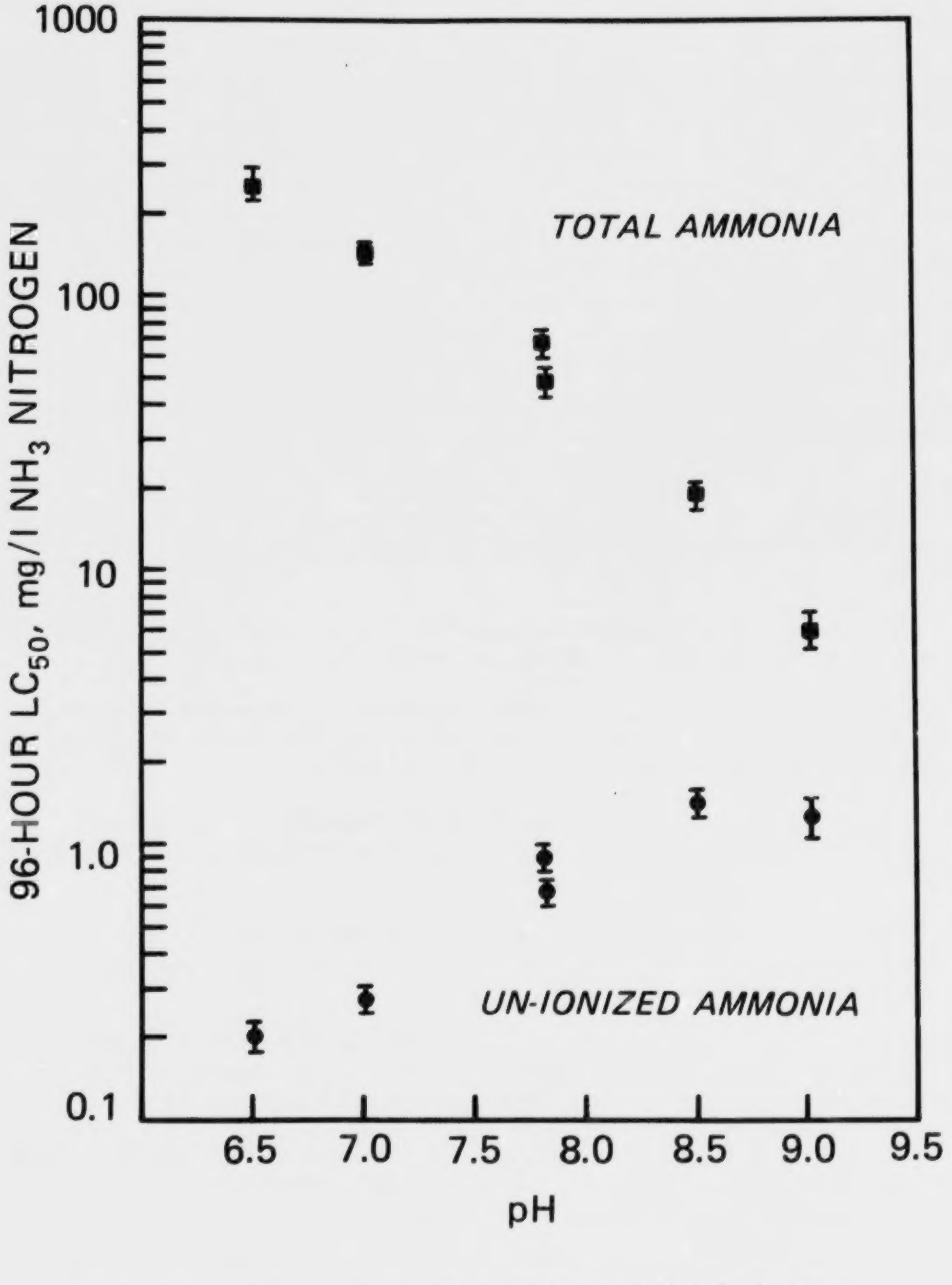

Figure 7. Acute toxicity of ammonia to fathead minnows: 96-hour LC50 vs. pH.

toxicity of a pollutant. Time limitations have necessitated a cursory treatment of both the published literature and the new research reported here. More complete information on this and other ammonia toxicity research being conducted both at Fisheries Bioassay Laboratory and at the Sunoga Laboratory here in Borok is now in preparation for journal publication in the Soviet Union and in the United States. The information I have presented illustrates some of the complexities involved in establishing water quality criteria and setting standards. It also underscores the necessity for continued collaborative effort between fish physiologists and water chemists, from laboratories such as ours and Sunoga, in conducting and interpreting the results of aquatic toxicity tests.

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SECTION 11

THE PREDICTION OF THE EFFECTS OF POLLUTANTS ON AQUATIC ORGANISMS BASED ON THE DATA OF ACUTE TOXICITY EXPERIMENTS

O.F. Filenko and E.F. Isakova[1]

The increasing number of pollutants requires acceleration of the ability to assess their toxicity, and to determine acceptable levels in the environment. These needs, coupled with a reduction of analytic costs, require a reduction in the length of experimental effort, and, at the same time, an increase in the reliability of the response.

To accelerate capabilities of assessment of toxicity, attempts were made to connect the biological activity of compounds with their physico-chemical properties. The correlation of toxicity of individual compounds with approximately 40 different physico-chemical properties were investigated (Filov and Liublina 1965). Naturally, a high correlation of these data for one organism is not sufficiently reliable for a group of species. It is known that reactions of different organisms, and occasionally even one organism, to the same toxin are different under altered conditions. In such cases, toxicity can differ by many orders of magnitude.

Another direction in the search has been an attempt to find the specific and especially sensitive reactions of organisms to the action of a given pollutant. These attempts have mostly failed. The sensitive and specific index for poisoning by land, an increasing level of 8-amino levulic acid in blood and urea, proved to be less sensitive than in the case of poisoning by mercury (Jackim 1973).

Usually such biophysical, biochemical, and physiological indices assist in identifying harmful effects after they have produced irreversible changes in the organism. The natural fluctuations of many of these indices in organisms are so wide that changes produced by chronic toxic action are usually unrecognizable. The picture is further complicated by the varying reactions of the organisms under the influence of toxic substances in varying environmental conditions.

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Thus, to be reliable, the index applicable to the rapid determination of biological effects of pollutants must take into account the peculiarities of both compounds and organisms. An example of one such approach to the problem can be found in the relationship of toxicity of organic in compounds in fish to values of their concentration gradients on the blood-brain barrier (Filenko and Parina, In press). It may be assumed that compounds of a homologous series have equally effective toxic potentials, but varying tissue accumulation capabilities, and that this is the principal reason for different resulting toxicity.

However, such general biological indices as survival and fecundity are still the most reliable. To decrease the time required for assessment of toxicity of a compound, instead of using the more reliable chronic experiments, acute toxicity tests of the compounds over a period of 24-96 hours usually are used. Application of such data for other conditions, concentrations, and species specific coefficients and factors can be used (Steinberg 1974). This approach is primarily useful as a quick screening methodology. When experiments are shortened, a portion of the reliability of response can be retained by increasing the number of experimental tests. Therefore, it becomes a question of the acceptability of the degree of simplification of conditions, and the reduction of the length of the experiment to that which is essential, and which involves a sufficient number of tests to make a reasonably reliable estimation of the probable effect of the material on the specific index in question for a period which exceeds the length of the time of observation.

An attempt to investigate aspects of this problem and some associated difficulties, are described in this paper. It should be noted, however, even the most carefully made predictions cannot equal the reliability of results from experimental verification.

METHODS

The experimental design utilized the water flea, Daphnia magna (Straus) in densities of 10 animals per 500 ml. The toxicity of individual compounds that are potential industrial and agricultural pollutants of water was assessed. The calculation of regression equations was made by the least squares method.

RESULTS AND DISCUSSION

The toxic effect of compounds on Daphnia was assessed by organism survival. The typical mortality curve for varying concentrations of compounds are shown in Figure 1. To demonstrate the regularity of this phenomenon, the coefficients for different equations that could describe the mortality of Daphnia in time were calculated. The results of such calculations for trimethyl tin chloride (TMTCh) are given in Table 1. The exponential, power, logarithmic, and parabolic functions were calculated. The fit of theoretical and experimental points was examined using correlation coefficients. The larger the coefficients, the greater the correspondence to a

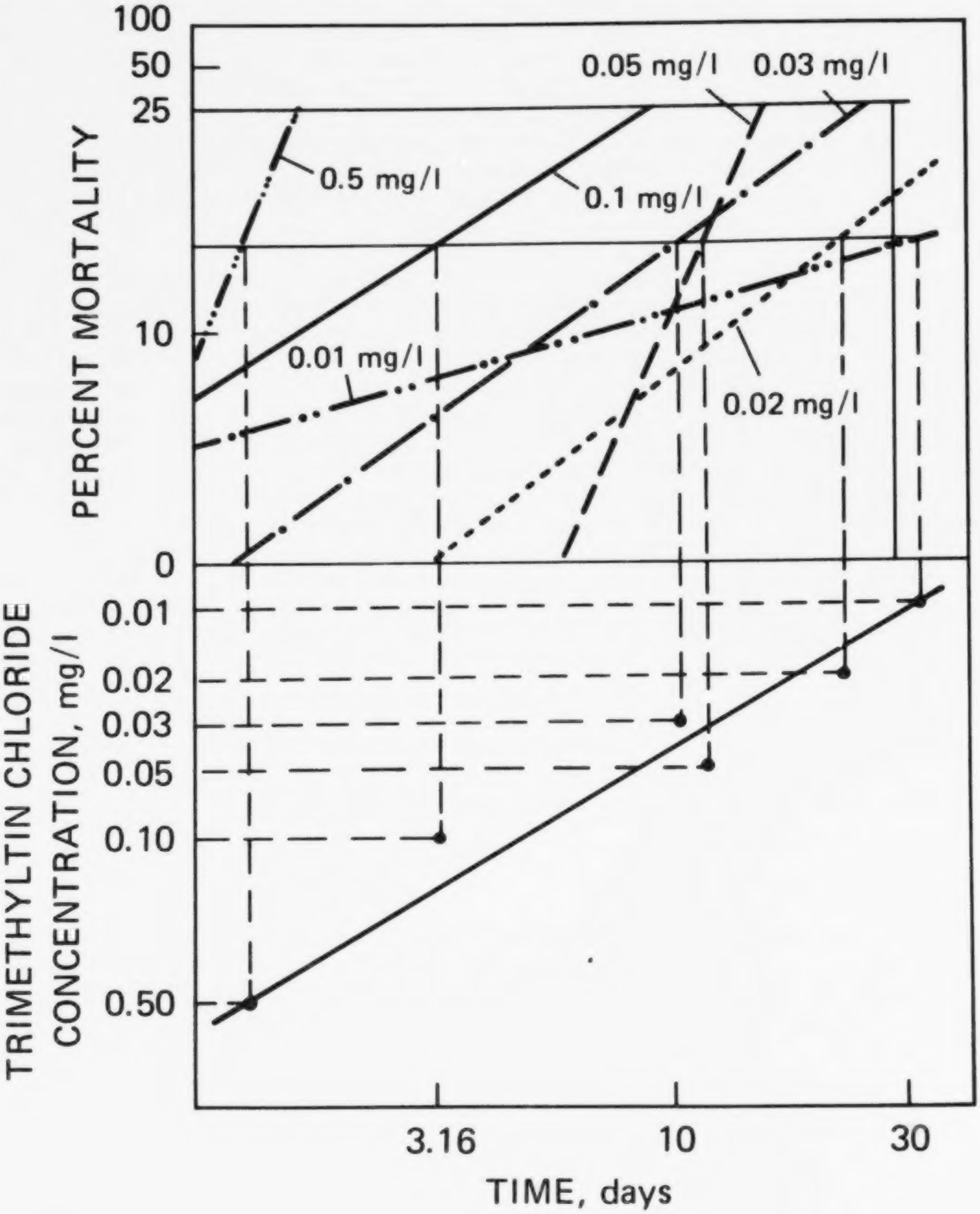

Figure 1. The relationship of the number of dead Daphnia magna (N) with time (T) under the influence of various concentrations of trimethyl tin chloride.

TABLE 1. DAPHNIA MAGNA RELATIONSHIPS OF PERCENT MORTALITY IN DAPHNIA MAGNA, AS CALCULATED BY VARIOUS EQUATIONS, WITH DURATION OF EXPERIMENT[1]

Type of Functions		Exponential			Power			Logarithmic		
Equations		$\ln N=\ln a + b\cdot T$			$\ln N=\ln a + b\cdot \ln T$			$N=a + b\cdot \ln T$		
Time (T) in days	Number of dead (N) in %	a	b	R	a	b	R	a	b	R
1	5									
3	25	2.24	0.805	1	5	1.465	1	5	12.2	1
6	35	4.8	0.36	0.89	5.56	1.12	0.96	5.4	16.88	1
7	80	4.5	0.396	0.94	5.22	1.273	0.97	0.06	29.93	0.84
8	90	4.71	0.381	0.95	5.09	1.32	0.97	-3.4	36.44	0.86
9	100	5.13	0.355	0.955	5.04	1.33	0.98	-6.35	40.94	0.97
The final form of equation		$\lg N = 0.71 + 0.8165\cdot T$			$\lg N = 0.124 + 5.04\cdot \lg T$			$N = -6.35 + 17.8\cdot \lg T$		

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[1] The concentration of trimethyl tin chloride - 0.1 mg/1.

high degree of fit. Equations for varying numbers of time observations from the start of experiment were calculated. A comparison of correlation values, calculated for different functions, shows that they are largest for power and parabolic functions, suggesting that these equations describe the regularity more accurately.

This conclusion was correct for varying concentrations of TMTCh. The power function lgN = a + b lgT, where N is number of dead Daphnia, in percentage, and T is the time in days, in logarithmic coordinates becomes a straight line (Figure 1), and it is possible to construct the curve based upon two points. Examples of the transformation of regularity of Daphnia mortality with time in logarithmic coordinates for organic tin and other compounds are given in Figure 2. It should be noted that the experimental and calculated values are not close enough. This fact is reflected by low values of correlation coefficients. It is possible that fluctuations depend on factors that are difficult to take into account in calculations, e.g., varying development of adaptive processes in organisms, and their altered reactions to environmental influences when exposed to different concentrations of compounds.

An attempt to analyze the dynamics of mortality in toxic solutions was made in order to understand the relationship of observed regularities to time. It is obvious for both groups of organisms, and for individuals, that they are influenced by the solution of toxic compounds, and that the toxic reaction increases through time, either as a function of continuous accumulations of the toxic materials, or as a result of the volume of alterations in the organism. The outcome for individual Daphnia will be the increasing of probability of death, and for a test group, there will be an increasing ration and rate of mortality. Thus, the slope of the curve increases dramatically in acute lethal experiments with organic tin compounds. In chronic studies, the curve progresses in a step-wise form. This reflects a sudden reduction in the rate of mortality with continuous exposure to toxic influences.

The explanation for this phenomenon lies in a combination or sum of two processes, (1) mortality under the influence of toxic substances, and (2) acceleration and enhancement of adaptive processes within the organism that inhibit mortality (Figure 3). The increase in toxicity proceeds more or less regularly with time, forming the basis for the adaptive processes that occur after the development of harmful effects in response to the toxins. It is not yet clear what activates these adaptive processes, the level of compound, the results of the deleterious effects in tissues, or the rate of increase of accumulation. It is possible to determine the rate of decrease or absence of mortality in toxic concentrations. Both of these two components, harmful effects and adaptation, can be described by adequate equations that can be used for further elementary analysis of the dynamics of the curve of mortality. However, the unique reactivity of living systems under the influence of toxic substances complicates the regularities that could describe the results of toxic effects. However, after calculating the coefficients a and b for the equation of power function, it is possible, with high degree of probability, to calculate the mortality of any percentage of Daphnia for a given period of time.

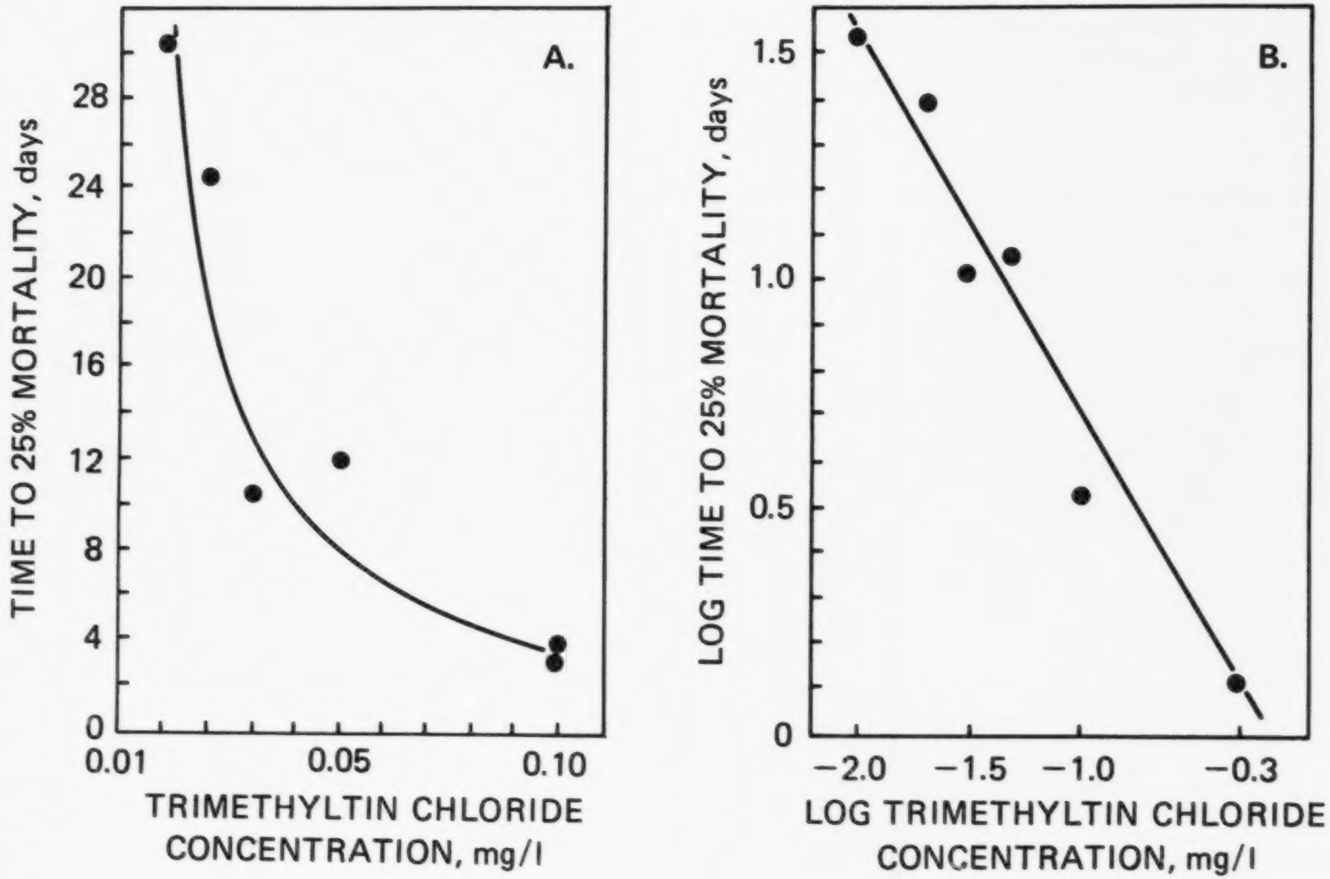


Figure 2. Daphnia magna mortality (N) with time (T) as a result of exposures to organic tin compounds (A), and some other compounds (B).

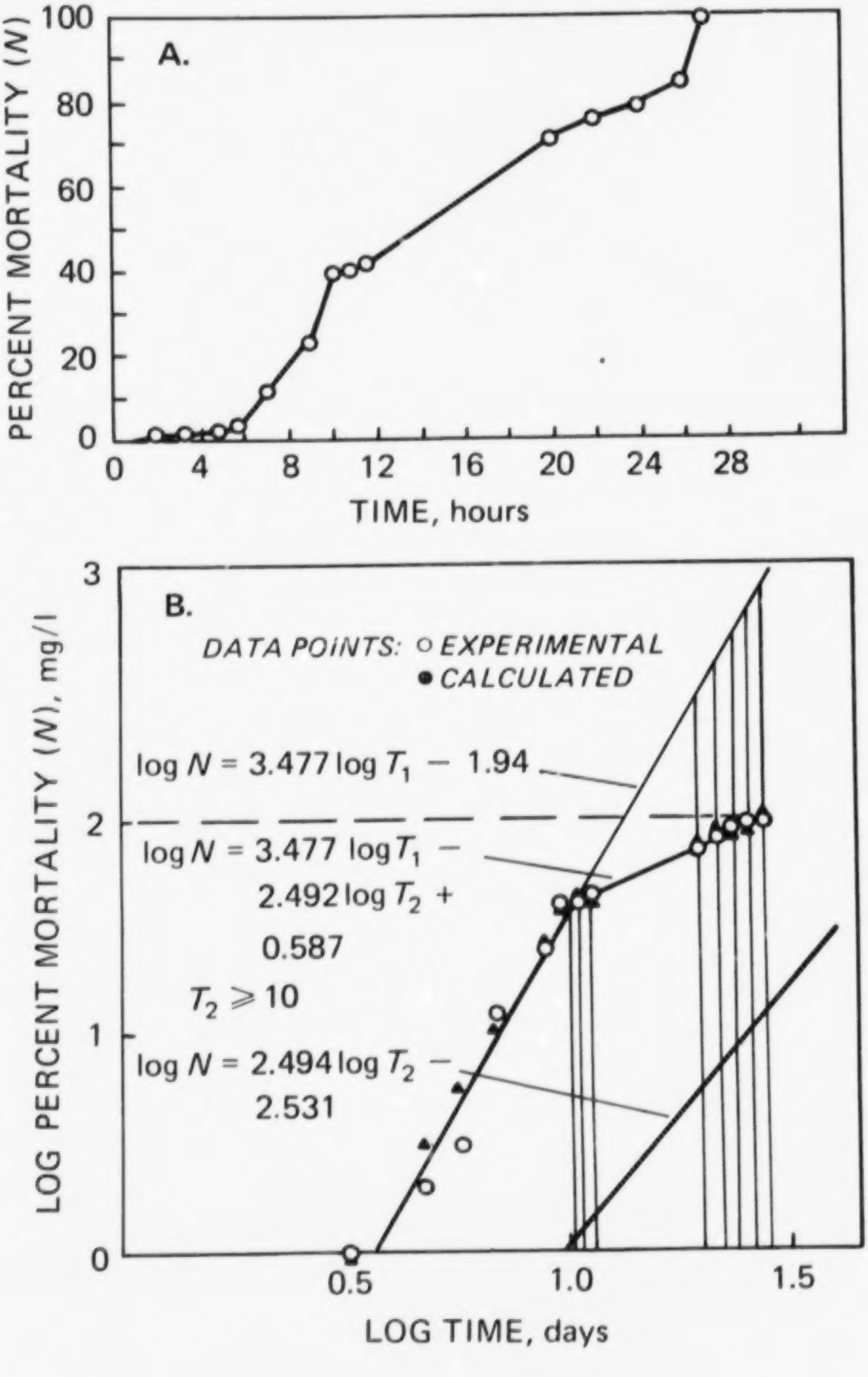


Figure 3. Daphnia magna mortality (N) with time (T) as a result of exposure to trimethyl tin chloride in a concentration of 1 mg/l, shown as (A) and as a logarithmic function (B). B. Regression equation plots for constructing a resulting line.

Stroganov (1975) recommends as acceptable the use of toxins that produce not more than 25 percent mortality. The equations present here calculate the data of death of 25 percent of Daphnia (T_{25}). As a rule, interpolations have been made, but extrapolation is also possible.

Practically, it is important to determine the minimum time period of observation that is sufficient for reliable calculations. To this end, regression equations for various time/mortality points were calculated. This enables a determination of the number of time points that would be sufficient for calculation of the T_{25}, value that does not differ significantly from the experimental value for 30 days. In Table 2 the dependence of correlation coefficients and the T_{25} value from the length of experiment is shown. The value of T_{25} does not significantly change for different periods of observation. This information makes it possible to limit the duration of experiments. For the calculation of coefficients for the equation, two dots are enough, but the reliability of calculated values will be low. For reliable results, it is advisable to have 3 to 4 time/mortality points for every concentration. In relatively high concentrations and with frequent recording of results, the time period can be very short. Thus, experimental results can be completed and quickly specify a preliminary assessment of acceptable concentrations.

As a result of these calculations a set of data is available that characterize the time of death of test organisms in varying concentrations (Table 3). The graphical relationship of concentration to time of death of 25 percent of Daphnia can be given as shown in the Figure 4A. This relationship can also be described by regression equations. From examined regularities (exponential, power, logarithmic and hyperbolic) the power function was found to be most suitable (Table 4). The correlation coefficients for the power function are highest, and it can be simply calculated by usual methods. This function is also suitable from a logical standpoint. Indeed, the curve of this function can never cross the axes, because time cannot be negative function, and there are enough small concentrations that do not influence the life-span of Daphnia. The concentration that does not effect Daphnia corresponds to the vertical asimptote.

There is certain diversity in the relationship of concentrations of pollutants to their effects (Warren 1971). However, these relationships can be described with a high degree of approximation by power or other simple functions. Using logarithmic axes, the power function becomes a straight line (Figure 4B), and approximate equation coefficients can be calculated from two concentrations.

By using these equations for certain compounds, we can evaluate the time of death for other concentrations, and estimate the concentration that causes the death of 25 percent of Daphnia in a given time period. The period of life-span can be limited to 30 days, and mortality to 25 percent. The concentration, that corresponds to these data, will be an acceptable concentration in terms of survival (Table 5). In this table the acceptable concentrations were calculated from data of concentrations, and a comparison with values that were accepted from experimental evidence is made. It is natural that there are some differences between experimental data and

TABLE 2. THE CORRELATION OF EXPERIMENTAL AND CALCULATED RELATIONSHIPS BETWEEN MORTALITY AND DURATION OF EXPOSURE OF DAPHNIA MAGNA TO TRIMETHYL TIN CHLORIDE USING VARIOUS EQUATIONS

Concentration (C) in mg/l	Time (T) in days	Number of dead (N) in %	lnN=a + b·T		lnN=ln a+b·ln T			N=a + b·lnT		lnN=a+b·T+d·T^2	
			R	T	R	P	T	R	T	R	T
0.1	1	5									
	3	25	1.00	3.00	1.00		3.00	1.00	3.00		
	6	35	0.89	4.48	0.97	0.10	3.83	1.00	3.90	1.00	3.00
	7	80	0.94	4.32	0.97	0.05	3.42	0.84	2.30	0.94	4.01
	8	90	0.95	4.38	0.94	0.01	3.34	0.86	2.30	0.96	3.98
	9	100	0.95	4.46	0.98	0.01	3.32	0.87	2.15	0.97	3.39
0.1	2	7.5									
	5	30									
	6	72.5	0.99	4.31	0.97	0.10	3.88	0.85	0.07	1.00	4.70
	7	95	0.99	4.34	0.97	0.05	3.81	0.87	2.96	0.99	4.32
	8	100	0.97	4.41	0.98	0.01	3.82	0.90	2.96	0.98	4.93
0.05	7	2.5									
	9	5									
	12	30	0.99	11.80	0.98	0.1	11.90	0.92	11.60	1.00	11.84
	15	80	0.99	12.17	0.99	0.01	11.81	0.91	9.87	0.99	11.94
	16	95	0.99	12.38	0.99	0.01	11.87	0.94	9.67	0.99	11.90
	17	100	0.98	12.60	0.99	0.01	12.00	0.95	9.64	0.99	11.82

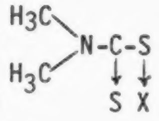
TABLE 3. THE DATE OF DEATH OF 25 PERCENT OF DAPHNIA MAGNA EXPOSED TO VARIOUS COMPOUNDS AS CALCULATED FROM EXPERIMENTAL STUDIES OF VARYING DURATION

Compounds				Concentration (mg/l)	First 3 points mortality			30 days of complete mortality		
Type	Chemical structure	Name	Abbreviation		Time (days)	T_{25}	R	Time (days)	T_{25}	R
ORGANIC TIN COMPOUNDS	$(CH_3)_3$ SnCl	Trimethyltinchloride	TMTCh	0.01	16	102.7	0.985	30	34.7	0.925
				0.02	13	16.1	0.992	28	24.4	0.952
				0.03	11	9.6	0.945	24	10.4	0.967
				0.05	12	11.9	0.978	17	12.0	0.992
				0.1	6	3.8	0.97	9	3.3	0.98
	$(C_2H_5)_3$ SnCl	Triethyltinchloride	TETCh	0.0001				30	34.5	1.0
				0.001	6	64	1	23	55.0	0.985
				0.01				29	78.0	0.982
				0.1	3	3.8	0.99	6	3.0	0.982
	$(C_3H_7)_3$ SnCl	Tripropyltin-	TPTCh	0.0001				30	43.8	0.9
				0.1	4	3.1	0.998	7	3.4	0.95
	$(C_4H_9)_3$ SnCl	Tributyltinchloride	TBTCh	0.0001	4	2.3	0.924	7	2.0	0.845
				0.001				6	4.2	0.996
	$(C_5H_{11})_3$ SnCl	Triamyltinchloride	TATCh	0.001	17	15.1	0.984	19	15.3	0.952
				0.01				6	4.8	0.958

TABLE 3 (CONTINUED)

Compounds				Concentration (mg/l)	First 3 points mortality			30 days of complete mortality		
Type	Chemical structure	Name	Abbreviation		Time (days)	T_{25}	R	Time (days)	T_{25}	R
ORGANIC TIN COMPOUNDS	$(C_6H_{13})_3SnCl$	Trihexyltinchloride	THTCh	0.002	22	43.0	0.98	27	43.3	0.988
				0.01	4	3.4	0.98	6	3.5	0.989
				0.1				2.6	0.4	0.999
				1.0	.2	1.1	0.956	1.8	1.1	0.809
	$(C_6H_5)_3SnCl$	Triphenyltinchloride	TPhTCh	0.01	14	11.3	0.98	19	10.8	0.985
				0.1	7	52.6	0.9	12	5.2	0.786
				1.0	0.6	0.4	0.99	2.1	0.5	0.935
				10.0				0.4	0.2	0.98
	$(C_4H_9)_2SnCl_2$	Dibutyltinchloride	DBTDCh water solut.	0.01				5	4.3	0.99
				0.1	7	6.4	0.997	10	6.7	0.96
				1.0	2	1.6	0.998	3.3	1.5	0.78
				10.0	0.6	0.4	1	1	0.4	0.98
			ethanolic solut.	0.001				30	49.3	0.98
				0.01	17	18.3	0.996	30	18.7	0.99
				1.0	1	0.9	0.911	2	0.9	0.95
	$(C_4H_9)_3Sn_2CH_6O_3$	Bis-(tributyltin) citrate	BTBTC water solut.	0.01				10	8.1	1
				0.1	6	4.4	0.947	6.5	4.1	0.94
				1.0	4	3.1	0.965	4.6	2.5	0.92
				10.0	0.8	0.3	0.997	0.8	0.3	0.99
			ethanolic solut.	0.001	7	6.8	0.996	29.0	9.0	0.95
				0.01	4.9	4.3	0.995	6.0	4.3	0.96
				0.1	0.8	0.3	0.997	1.0	0.2	0.97
				0.5				0.5	0.2	1

TABLE 3 (CONTINUED)

Compounds				Concentration (mg/l)	First 3 points mortality			30 days of complete mortality		
Type	Chemical structure	Name	Abbreviation		Time (days)	T_{25}	R	Time (days)	T_{25}	R
FUNGICIDES	Piror-400 (Commercial Name)			1.0	9	10.1	1	26	30.4	0.8
				10.0	5	3.3	0.958	30	3.1	0.77
		Dimethylditiocarbamates X: Ca - 6 mg/l Mg - 3 mg/l	"Mixture I"	0.009	14	29	0.998	30	35.4	0.99
				0.09	14	15.9	0.981	30	20.5	0.96
				0.9	3	0.3	0.957	4	0.44	0.92
				9.0	3	1.5	0.992	4	1.5	0.99
		Ca - 2 mg/l NH_4 - 2 mg/l Mg - 0.4 mg/l	"Mixture II"	0.0044	21	37.4	0.977	30	33	0.99
				0.044	17	18.4	0.97	30	38.4	0.83
				0.44	3	3.9	0.96	7	3.3	0.99
				4.4				4	2.7	0.99
SALTS	$MnSO_4 \cdot 7H_2O$	Manganese sulphate		0.01	21	19.7	0.999	30	21	0.94
				0.1	13	3.1	0.998	28	7.8	0.98
				1.0	10	22.8	0.999	18	13.3	0.95
				10.0	13	12.8	0.91	23	14.7	0.93
				50.0	1.3	0.7	0.96	1.9	0.7	0.98
	$MnSO_4 \cdot 7H_2O$	Magnesium sulphate		1.0	20	17.4	0.988	30	21.5	0.82
				10.0	16	12.5	0.992	20	12.4	0.99
				100.0	21	5.5	0.882	30	3.6	0.92
				1000.0	2.1	0.7	0.996	3.7	0.8	0.97

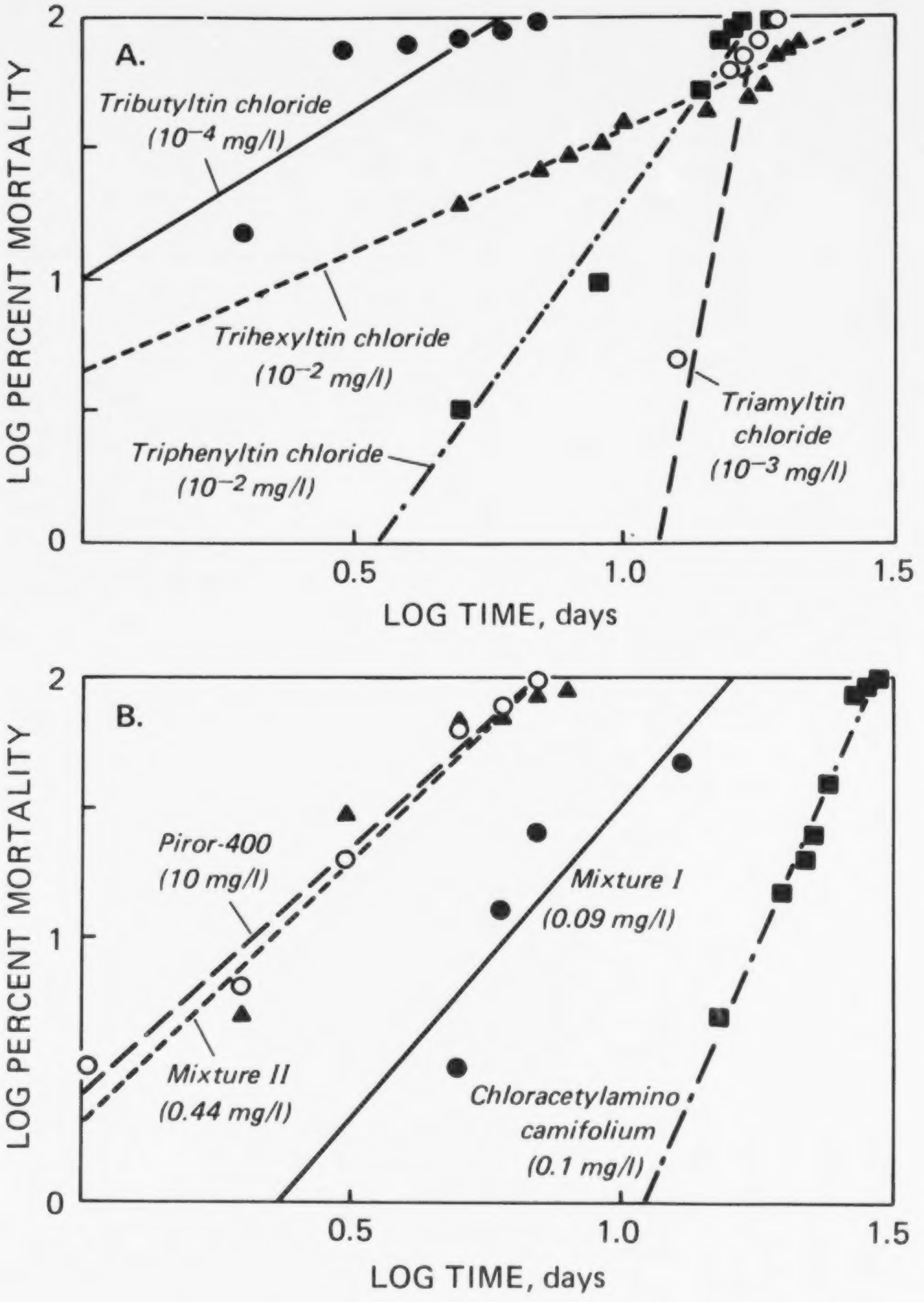


Figure 4. The relationship of time of death of 25 percent of Daphnia magna with the concentration of trimethyl tin chloride.

TABLE 4. THE RELATIONSHIP OF THE TIME OF DEATH OF 25 PERCENT OF *DAPHNIA MAGNA* WITH CONCENTRATIONS OF TRIMETHYL TIN CHLORIDE CALCULATED BY DIFFERENT FUNCTIONS

Type of Functions		Exponential			Power			Logarithmic			Hyperbolic		
Equations		$\ln T_{25}=\ln a+bC$			$\ln T_{25}=\ln a+b\cdot\ln C$			$\ln T_{25}=a+b\cdot\ln C$			$T_{25}=a+b/C$		
Concentration (C) in mg/l	Time (T) in days	a	b	R	a	b	R	a	b	R	a	b	R
1	0.5												
0.5	1.3												
0.1	3.32	3.95	-2.1	-0.99	0.59	-0.78	-0.97	-0.48	-1.23	-1.00	0.45	0.29	0.98
0.05	12	7.32	-2.85	-0.93	0.34	-0.95	-0.98	-0.60	-3.26	-0.86	-0.51	0.58	0.97
0.03	10.37	8.51	-3.04	-0.94	0.57	-0.88	-0.98	1.61	-0.60	-0.90	0.86	0.35	0.89
0.02	24.43	11.40	-3.42	-0.01	0.55	-0.92	-0.98	-2.24	-4.88	-0.84	-0.07	0.45	0.98
0.01	34.67	14.51	-3.74	-0.90	0.56	-0.91	-0.99	-4.57	-6.58	-0.87	1.41	0.35	0.97

TABLE 5. ACCEPABLE CONCENTRATIONS OF COMPOUNDS FOR SURVIVAL OF DAPHNIA MAGNA CALCULATED WITH EQUATIONS OF POWER FUNCTION

Compound	Maximal acceptable concentrations (mg/l)	
	Determined in chronic experiments	Calculated on the data of acute toxicity
TMTCh	0.01	0.02
TETCh	0.01	0.02
TPTCh	0.001	0.0002
TATCh	0.0005	0.0003
THTCh	0.002	0.001
TPhTCh	0.01	0.003
DBTDCh	0.001	0.005
Bis(THT)cytrate	0.0001	0.00026
Piror-400	1	1
"Mixture I"	0.009	0.011
"Mixture II"	0.044	0.01
Manganese sulphate	0.01	0.005
Mangnesium sulphate	1	0.85

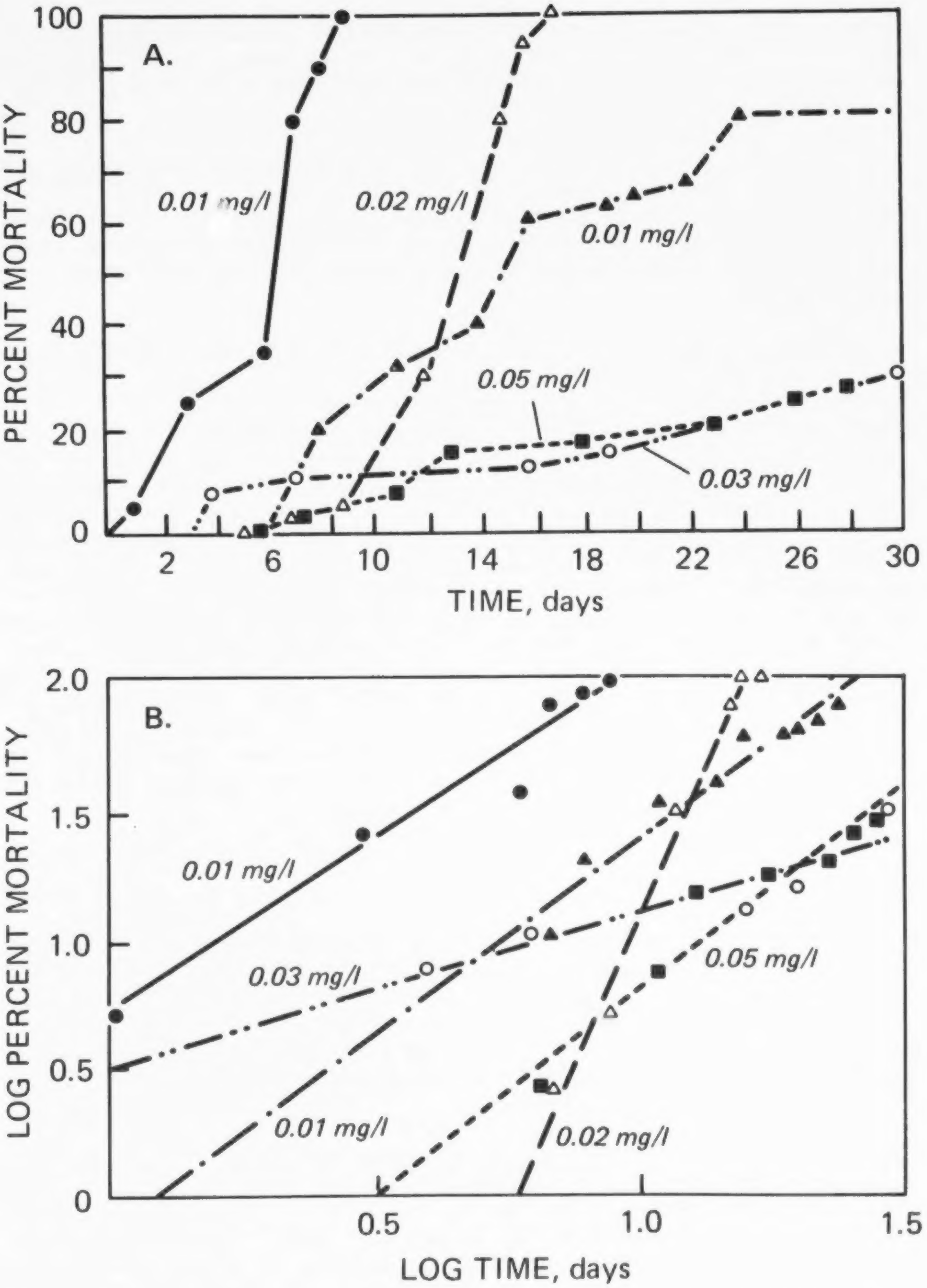


Figure 5. Graphical determination of acceptable concentrations of trimethyl tin chloride for Daphnia magna.

theoretical data. However, even in experimental determinations there may be a diversity in repetitions, as well as deviations caused by the toxicological experiment itself, especially when the test concentrations utilized differ by orders of magnitude.

The reported results were derived for in experiments on *Daphnia*, but this approach is applicable for other aquatic organisms as well. This approach has been shown to be particularly effective in experiments with long-lived species (Parina *et al*. 1979). It is assumed that these regularities are applicable for other indices of the effects of toxic substances on aquatic organisms.

In summary, it may be concluded that of all newly developing methods of quick screening of toxic effects of pollutants on the aquatic organisms using forecasting techniques, the most effective method, is still the use of mathematical extrapolation of data from acute experiments. The dynamics of the results of toxic influence for aquatic organisms (mortality) can be shown as a combination of simpler processes. The connection of mortality with time, and the onset of given effects with concentration can best be described by power function equations. For evaluation of regression equations describing these statistically reliable relationships, 3 to 4 experimental points are necessary. These equations can be used for determination of the effects of the pollutant on the organisms for a period that exceeds the duration of observation, and for concentrations, that have not been experimentally investigated, including an approximation acceptable concentrations of the pollutant in the aquatic environment. It is particularly advisable to use this approach for work under field conditions, or with long-lived organisms, when the possibility of long-term observations does not exist. This approach may also be used for investigations into a wide spectrum of concentrations of a given pollutant.

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SECTION 12

AGE SPECIFICS OF SENSITIVITY AND RESISTANCE OF FISH TO ORGANIC AND INORGANIC POISONS

V.I. Lukyanenko[1]

The continually increasing interest of researchers in the age aspects of toxicoresistance of fish (Mironov 1972; Kuhnbold 1972; Eisler 1972; Mitrovic 1972; Shmalgauzen 1973; Samylin 1974; Waldiehuk 1974; Danilchenko 1975; Dethlefsen 1975; Patin 1977, etc.) results from many factors, two of which are particularly interesting.

1. The first is the need to understand the paths of direct toxic influence of various substances entering the water on ichthyofauna and, in the final analysis, on the productivity of the reservoir. As we know, toxic substances affect all stages of the life cycle of fish: from fertilization of eggs to sexually mature individuals. However, from the ecologic standpoint, the early stages of ontogenesis of fish (embryonal and immediate postembryonal) are most vulnerable from the standpoint of the toxic factor, since they cannot actively migrate and avoid polluted water. It follows from this that the reaction of a population of fish to chemical pollution will be determined by the effect of the toxic factor on these early stages of ontogenesis if they are less resistant than mature fish.

2. The second factor determining the activation of research in the area of the age factor in ichthyotoxicology is the search for the most vulnerable stage in individual development of various species of fish, which should be used as the test object in the determination of the basic parameters of toxicity of various groups of substances and subsequent determination of maximum permissible concentrations (MPC) for these substances. It is quite understandable that the least resistant stages of ontogenesis development of fish are of primary interest for those involved in development of the problem of biologic testing of the quality of natural and waste waters.

The possible influence of pollution on larvae and fry was first mentioned in the last century. For example, the great Russian ichthyologist,

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O.A. Grimm (1896), in his now classical monograph, "Kaspiysko Volzhskoye Rybolovstvo" (Fishing the the Caspian and Volga), in analyzing the paths of influence of petroleum on the "fish content" of this basin, wrote, "It is quite probable that petroleum kills the fry of the Clupeidae family of fish and others, which float on the top or accumulate near the bank in shoals". Somewhat later, H. Clark and G. Adams (1913) concluded that one of the leading causes of the decrease in the population of whitefish was pollution of the spawning grounds in the Great Lakes with industrial wastewater. However, experimental study of the age specifics of toxicoresistance of fish began only comparatively recently.

One of the first reports in this area is that of N.S. Stroganov and A.M. Pazhitkov (1941). In experiments with eggs, larvae, fry and mature individuals of perch, it was shown that the early stages of development are less resistant to the ions of copper and ammonia than mature fish. Given equal exposure, mature perch survived in solutions of copper 100 times more concentrated than the lethal concentration for fry. In experiments with ammonia, the difference was less striking, but still clearly indicated the lower stability of embryos and perch larvae than that of mature fish.

The high resistance of mature fish in comparison to larvae and fry for heavy metal salts was noted by other authors as well (Sollman and Schweiger 1957; Cairns and Seheir 1957). However, in later works, materials have been presented indicating that the stability of fish in the early stages of ontogenesis is higher than that of mature individuals, or at least equal (Mosevich, et al. 1952; Wurtz-Arle 1959; Katz and Chadwick 1961; Vernidub 1962). For example, N.A. Mosevich, et al. (1952), in experiments with eggs, larvae and first-year perch, established that the first-year fish were less resistant to phenol than the eggs and larvae. Developing eggs and recently hatched larvae were found to be more resistant than mature fish to the pesticide andrin (Katz and Chadwick 1961). These data agree with the materials of Ye.A. Veselova, et al. (1965), who studied the toxicity of still another pesticide - hexachlorane - and concluded that developing eggs and larvae of many species of fish (salmon, roach, bleak, perch, rock perch, pike) are somewhat more stable than mature individuals. Finally, in a work of D. Wurtz-Arle (1959) performed on developing eggs and fry of trout, it was shown that their resistance to two detergents (sodium alkylsulfates) decreases with age.

Thus, in the mid-1960's there were two mutually opposite points of view. The proponents of one believed that "the most vulnerable stage of ontogenesis in fish for the effects of toxic substances is the stage of the larvae and fry" (Stroganov and Pazhitkov 1941, p. 68), i.e., the toxicoresistance increases with age. The other group of authors held the opposite point of view, assuming that the resistance of fish to poisons decreases with age and that it is highest in the early stages of ontogenesis.

Analysis of the available literature data has allowed us (Lukyanenko 1967) to find the reasons for this contradiction. It was found that the proponents of the idea of increased stability of fish in early stages of individual development based their ideas on data obtained in experiments with organic poisons (phenols, synthetic detergents, pesticides). Researchers holding the opposite point of view, that of reduced resistance of fish to poisons in the early stages of ontogenesis, had performed experiments with inorganic poisons, primarily heavy metal salts. This indicated to us that the seeming disagreement, concerning the level of toxicoresistance of various stages of ontogenesis of fish, resulted in fact from the different nature of the toxic substances studied and, consequently, the differences in mechanism of action of the poisons, organic and inorganic in nature, on the developing eggs, larvae and fry.

Considering the importance of this problem, both in the theoretical and in the practical aspects, we undertook an experimental test of this assumption, concentrating our emphasis on organic poisons. Since in most works on the age variation of ichthyotoxicology, authors have used some single "point" of embryonal, larval or fry development, we decided to study the dynamics of toxicoresistance in each of the three periods of early ontogenesis. In our report, we summarize the results of many years of studies performed on bony fishes (rainbow trout, bream, zope, carp) and cartilagenous ganoids (Russian sturgeon, Caspian sturgeon, sterlet and giant sturgeon). The toxins used represented a broad range of concentrations of phenol, certain pesticides (metaphos, yalan and propanid), as well as chlorides of cadmium and cobalt, in order to determine the age specifics of toxicoresistance of the fish to inorganic poisons.

In our initial experiments, performed jointly with V.M. Volodin and B.A. Flerov on the eggs, larvae, fry and mature individuals of two systematically similar species of the genus *Abramis*; the bream (*A. brama*) and zope (*A. ballerus*), exposed to the toxic effects of 12 different concentrations of phenol (from 1 to 5000 mg/liter), we found that the toxicoresistance of mature fish was significantly lower than that of the eggs, embryos and larvae (Volodin, *et al*. 1965, 1966). This was reflected both in the lethal concentrations for fish of the various age groups, and in the time of survival of each of the age groups studied with identical or similar concentrations of toxic substance.

The decrease in resistance of fish to phenol from young age groups to older age groups agrees with the available data from the literature; however, in these same experiments we found that, within each of the three main stages of early ontogenesis; embryonal, larval and fry, toxicoresistance undergoes significant changes. For example, the least stable period of embryongenesis was found to be the earliest - from the beginning of division to the formation of the embryo, particularly the stage of gastrulation. Beginning with the early formation of the embryo, resistance to phenol greatly increases. Suffice it to say that, with a phenol concentration of 100 mg/liter, zope eggs in the early stages of development die 8 times more rapidly than in the stage of formation of the embryo. After emergence from the shell, resistance of the embryos decreases greatly and embryos without shells die in half the time as those still in the shells. The significant decrease in the resistance of embryos after hatching from the shell indicates the great significance of the shell, preventing penetration of the poison and its accumulation in the organisms during the embryonal period of development.

During subsequent ontogenetic development, resistance of fish to phenol continues to drop. The survival time of zope larvae in the stage of mixed feeding in phenol solutions of 100 and 150 mg/liter was found to be 48 and 30 hours, respectively. This is 1/5 the time of survival of the embryos in the stage of beginning of pulsation of the heart (240 hours) and 1/2 the time of survival of hatched embryos. Whereas, during the embryonal period of development, the toxicoresistance of the zope undergoes significant changes throughout the entire larval period of development; i.e., at the beginning, middle and end, it remains more or less at the same level. Then, in the early fry period of development, the stability of the zope to phenol drops greatly (by a factor of more than 10) and the mean survival time in phenol solutions of 150 and 100 mg/liter becomes 2-3 hours. However, the least resistance was noted for mature zope, which survived only 6-8 hours in a phenol solution of 25 mg/liter, i.e., 1/4-1/6 the concentration used in the experiments with the fry. Let us recall that the eggs, embryos and larvae survive and develop without any significant deviations from the norm in a solution of this concentration. In order to cause death of eggs in this same time interval, the concentration of phenol must be increased to 1000 mg/liter, i.e., by a factor of 40.

Thus, the resistance of the zope in the early stages of ontogenesis to one of the most widespread organic poisons, phenol, undergoes significant changes. The least resistance is that of the eggs in the stage of gastrulation; the greatest, that of the eggs in the stage of pulsation of the heart. Subsequently, the level of toxicoresistance decreases continually from hatching embryo to larvae, from larvae to fry and fry to adults. An analogous variation was observed in experiments with eggs, hatched embryos, larvae, fry and mature individuals of another species of the genus Abramus, the common bream.

In experiments with still another species of carp (Carassius carassius), we succeeded in comparing the toxicoresistance of four age groups: current year's brood, 1-, 2- and 3-year fish (Lukyanenko and Flerov 1963). The criterion of resistance was the time of survival of experimental fish in toxic solutions of phenol (17-800 mg/liter). As was to be expected, the most resistant carp was the current year's brood, which survived many times longer than older fish. For example, in a phenol solution of 50 mg/liter, the mean survival time of the current year's brood was 137.4 hours, of fish 1-2 years old - 34.9 hours, of fish which had completed 2 years of life - 12.4 hours, of fish over 3 years old - 5.7 hours. Analysis of these materials indicates that the survival time of the current year's brood in comparison to carp 1+ years old is 3.9 times greater, than that of carp 1+ years old in comparison to carp 2+ years old 2.8 times greater. The difference between the next two age groups (2+ and 3+ years) is still less, a factor of 2. The impression is gained that, as age increases, the resistance of the fish, after reaching a certain level, undergoes only moderate changes. However, there is no doubt that fish in the younger age groups are more resistant to phenol than fish in the older age groups.

This is also indicated by the results of a comparative study of the level of toxicoresistance of the current year's brood and two-year-old rainbow trout (Salmo irideus Gibb) which we performed (Lukyanenko and Flerov

1966) using the phenol intoxication model. The elevated resistance of the current year's trout brood in comparison to 2+ year old individuals was reflected both in the absolute values of CLM (minimal lethal concentration), CMT (maximum tolerant concentration) and LC_{50} (concentration causing death of 50% of experimental fish), as well as the mean time of survival at all concentrations of phenol tested (5, 7.5, 12.5, 15, 20, and 25 mg/liter). In experiments with the current year's brood, the CLM was 15 mg/liter, LC_{50} - 11 mg/liter, CMT - 7.5 mg/liter, while in experiments with 2+ year old fish the figures were 10 mg/liter, 7.5 mg/liter and 5 mg/liter, respectively. The differences between two age groups of trout in terms of time of survival at a given concentration of phenol were still more sharply expressed. The mean time of survival of two-year-old trout in a phenol solution at 12.5 mg/liter was only 95 minutes, i.e., less than 1/6 the survival time of the current year's brood - 601 minutes. No less demonstrative were the differences found in comparison of times of survival of the current year's brood (272 minutes) and two-year-old fish (40 minutes) in a solution of 15 mg/liter phenol, survival being almost 7 times longer for the current year's brood.

The increased resistance of younger age groups, which we found in our experiments with phenol in highly resistant carp and more susceptible trout, indicates that what we have here is a general regularity of reactions of fish of different levels of organization to organic poisons. In order to test this assumption, we performed experiments (Kokoza 1970) on fry, 35-70 days of age, of three species of sturgeon: the Russian sturgeon, Caspian sturgeon and sterlet, representing the evolutionarily more ancient group of cartilagenous fish. The experiments involved phenol at 50 mg/liter. We will not take the time to present the results of this series of experiments in detail, but rather shall note only the clearly expressed specific differences in the level of toxicoresistance, manifested in the fry period of development. The mean survival time of 40-45 day old fry of Russian sturgeon (12 hours 24 minutes) was 4 times greater than that of sterlet fry of the same age (3 hours 05 minutes), and 2.6 times greater than that of Caspian sturgeon of the same age (4 hours 40 minutes). Sexually mature Russian sturgeon, which survived in a phenol solution of 40 mg/liter for 5 hours 30 minutes, were also characterized by higher toxicoresistance in comparison to the Caspian sturgeon (1 hour 20 minutes) and sterlet (1 hour 35 minutes) (Lukyanenko 1967).

However, in this case, we would like to concentrate our primary attention, not on the specific differences of toxicoresistance of the sturgeons during their fry period of life, but rather on age differences, i.e., to compare the time of survival of mature individuals of each of the three species and 1-2 month fry of the same species. This comparison showed clearly that the resistance of mature fish, as indicated by survival time in phenol solutions of similar concentrations (40 and 50 mg/liter), is only 1/2 to 1/3 the resistance of fry. In other words, the conclusion which we have reached, that of decreasing level of resistance of fish with increasing age in terms of organic poisons, is true not only for the evolutionarily young and highly organized bony fish, but also for the cartilagenous fish, lower on the evolutionary scale.

The materials, which we have accumulated in our laboratory in the past 10 years, indicate clearly that the resistance of various groups of fish to poisons differs in different stages of ontogenesis. Periods of high stability (eggs in the stage of pulsating heart, larvae in stage C_2 and current year fish) alternate with periods of low resistance (eggs in the stage of gastrulation, fry in their early period, sexually immature individuals). Particular attention should be given to the end of the larval period of development and the beginning of the fry period, when toxicoresistance drops sharply, approaching that of mature individuals, or falling somewhat below it. On the whole, however, the resistance of various species to organic poisons decreases with continuing ontogenetic development and reaches its minimum in mature individuals. We relate this fact, observed repeatedly in our laboratory, to the formation of various functional systems in the organism in ontogenesis and their neurohormonal control, which determines the level of reactivity of the entire organism to various physical and chemical irritants. An important role should be paid by the central nervous system and its synaptic structures, since the toxic effects of many poisons in the organic series are manifested by disruption of this activity, and consequently dysfunction of the basic physiologic systems (Lukyanenko 1967).

This point of view is held by a number of domestic researchers. In the opinion of O.I. Shmalgauzen (1973), the younger stages of sturgeons (Caspian sturgeon and Russian sturgeon) are more resistant to phenol than larvae as they go over to active feeding. Whereas, a phenol concentration of 40 mg/liter is sublethal for eggs and only the teratogenic effect of phenol is manifested, for larvae which have begun active feeding this concentration of phenol is lethal. Larvae die with symptoms of acute phenol poisoning, described for mature fish by O.I. Shmalgauzen (1973), indicating the "phenol acts" on the larvae as a poison specifically damaging the nervous system (page 7).

An objective study of the resistance of various species of fish in early ontogenesis to certain toxins was undertaken by A.F. Samylin (1974). Comparing the resistance of Salmo salar to ammonium carbonate during various periods of ontogenesis, he came to the conclusion that as the eggs of the fish increased, the survival time in the same concentrations of the substance decreased. A similar picture was observed in experiments with urea (carbamide): Fry were less resistant to this toxin than eggs and larvae. The decrease in resistance of salmon with increasing age observed in this experiment was also seen in experiments with three pesticides; hexachlorane, pentachlorophenol and copper naphthenate. We must note that the toxins used in this work differ significantly in their mechanism of action and a number of other properties, particularly their cumulative properties. Whereas ammonium carbonate is a physiologically cumulative poison, hexachlorane is a materially cumulative poison. Nevertheless, a decrease in toxicoresistance with increasing age was observed in experiments with all of the substances. Summarizing the results of the experiments, performed with five different toxins, differing greatly in their degree of toxicity, the author emphasizes that as ontogenetic development continues, the resistance of the salmon decreases. In complete agreement with our earlier published data on the age dynamics of the resistance of fish to phenol (Lukyanenko 1967), A.F. Samylin (1974), concludes that there is a significant change in the level of toxicoresistance during various periods of ontogenesis. The least resistance was noted in salmon in the stage of gastrulation in the embryonal period of development, during transition of larvae to active feeding in the larval period of development and during transformation of larvae to fry, i.e., in the early fry period of development. These "points" of decreased resistance of each of the three stages of early ontogenesis, found in experiments on salmon with various toxins, are identical to those which we found in our experiments (Volodin, et al. 1966) with phenol using the eggs and larvae of the zope and bream.

Thus, at the present time there is sufficient proof of increased resistance of the early stages of ontogenesis, primarily the embryonal period of development, to organic poisons. The materials of a number of authors, indicating that the resistance of fish in the early stages of ontogenesis to organic poisons is significantly less than that of mature fish, are not in agreement. For example, according to S.A. Patin (1977), developing eggs and particularly larvae of the Stauridae are hundreds of times less resistant to the effects of polychlorinated biphenols than are mature fish of related species. He also noted higher resistance of embryonal and larval periods of development in comparison to mature individuals in experiments with other organic poisons, petroleum and surfactants. Recalling that these data do not agree with many reports in the literature on the elevated resistance of the embryonal period of life of fish obtained, true primarily with fresh-water forms or transient forms, S.A. Patin assumes that one reason for the disagreement is the salinity of the medium, which may change the toxic properties of detergents. We can add to this the fact noted earlier (Lukyanenko 1967) of decreased resistance of sea fish in comparison to fresh-water fish which, apparently, is true for all stages of individual development.

Still, it is difficult to understand the reasons for the reduced resistance of the embryonal period of life in comparison to later stages of ontogenesis to organic poisons. However, increased toxicoresistance in the early stages of ontogenesis, in our opinion, is quite easily explained. As we know, fish embryos in the early stages of development are protected by the egg shell, which is an effective barrier for foreign substances, including toxic substances (Skadovskiy 1955). This factor causes the unique conditions of influence of organic toxins on the embryonal stage of development of fish. No matter how toxic a substance dissolved in water may be, in order to manifest its toxicity it must penetrate the egg shell and reach the perivitelline fluid. The toxic effect is a function of concentration of the substance and time of action. Therefore, it can manifest its action only if a quantity of the substance accumulates in the egg sufficient to influence the metabolic processes of the embryo and, in the final analysis, the course of morphogenesis. It follows from this that the more difficult it is for a substance to penetrate the egg shell, the less toxic it is for the embryo still in the egg. Therefore, we must realize that in those cases when we record increased resistance for the embryonal period of development of fish to organic poisons, it is determined not only by the fact that the substance has little influence on the metabolism of the developing organism, but also the fact that the concentration of the substance penetrating through the egg shell into the perivitelline fluid is significantly lower than that dissolved in the water. Quite understandably, we can determine the true causes for increased resistance of the embryonal period of life of fish to toxins only if we have information on the concentration of the substance not only in the water, but also within the egg. Of course, it is difficult to produce this information, but the first studies in this area (Rosenthal and Sperling 1974; Dethlefsen, et al. 1975; Rosenthal, et al. 1975; Westernhagen and Dethlefsen 1975; Patin 1977) confirm the existence of a relationship between manifestation of the toxic effect and the degree of permeability of the egg shell. True, most works have been performed with inorganic poisons, with heavy metals, and particulary with cadmium. It has been found that the egg shell can form strong complex bonds with the metal, thus preventing its penetration to the embryo (Rosenthal and Sperling 1974; Westernhagen and Dethlefsen 1975). The thicker the shell, the greater the supply of active centers bonding the metal and the greater the quantity of metal it can accumulate. However, the coefficients of accumulation of metal by the larva are determined not only by the morphophysiologic properties of the shell, but also by the physical-chemical status of the metal in the water. Ionic and molecular forms of zinc and copper, which easily form strong complexes with biologic substrates, have a higher coefficient of accumulation than cadmium and particularly lead, which are more frequently present in hydrolyzed and suspended form in the marine medium. We can agree with the opinion of those authors, who believe (Patin 1977) that adsorption of a metal onto the egg shell does not mean that it has penetrated to the embryo. Such metals as lead or cadmium, bonding firmly with the active centers in the shell, apparently find it considerably more difficult to penetrate into the shell than the easily soluble ionic forms of zinc or copper. These two latter metals can penetrate into the perivitelline fluid and accumulate in the embryo. Based on the concept of increased vulnerability of the early stages of ontogenesis for toxic substances as a whole, and heavy metals in particular, the resistance of the eggs to zinc and copper should be lower than the resistance of the larvae. However, according to the information of Skidmore (1974), the eggs of fish are 20 times more resistant to the toxic effects of zinc than are the larvae, while the toxic effect of copper, which also easily penetrates the shell barrier, is approximately the same for eggs and larvae (Patin 1977). It follows from this that even with respect to inorganic poisons (metals), the idea of decreased toxicoresistance of the embryonal period of life requires some significant adjustment, for two reasons:

First of all, the available factual data indicate that eggs are not less resistant to all inorganic poisons than, say, the emerging prelarvae and larvae (Skidmore 1974; Patin 1977, Bengtson 1974; Blexter 1977). Secondly, and this is particularly important, the high specific surface of embryonal and postembryonal stages of development of fish, which are small in this period, should lead to accumulation of higher concentrations of the toxic substance (if they penetrate the biologic membranes) than, e.g., in larger individuals of the same species in later stages of development. In any case, the radioecology of fish provides us with data indicating the presence of some feedback between the specific surface of hydrobionts, including fish eggs, and the intensity of accumulation of radioactive substances. The smaller the dimensions of the hydrobiont and, consequently, the greater the surface of contact with the surrounding medium, the higher the concentration of the toxic substance in the organism. In order to conclude reduced resistance of eggs in comparison to larvae or, say, fry, we must compare their survival time at various concentrations of toxin actually penetrating into the organism. Therefore, any author stating that fish eggs have reduced resistance of so-called increased sensitivity must present data on the concentration of the toxic substance in the developing organism. Unfortunately, such data have not yet been presented.

As concerns the statement, sometimes seen, of increased vulnerability or reduced resistance of eggs to organic poisons, they simply do not agree with the multitude of factual data accumulated at the present time in both the domestic and foreign literature (Bandt 1948; Mosevich, et al. 1952; Wurtz-Arle 1959; Katz and Chadwick 1961; Veselov 1965; Volodin, et al. 1966; Lukyanenko 1967; Samylin 1974; Danilchenko 1975; Hakkila and Nilmi 1973; Wilson 1976; Wienberg 1977; Paflitscher 1976).

The increased toxicoresistance of developing eggs to organic poisons can be easily understood if we keep in mind that most of these substances cannot penetrate the shell or penetrate very slowly, so that is is difficult for them to reach effective concentrations inside the shell. Thus, according to S.A. Patin (1977), the lethal concentration (LC_{50}) of polychlorinated biphenyls are 8 times less for developing fish eggs than for larvae, which the author correctly relates to the inability of these substances to penetrate to the embryo through the egg shell. In earlier observations, H. Bandt (1949) noted increased resistance of larvae to hexachlorane, which was present at 2.5 mg/liter, many times greater than the lethal concentration for mature roach, his test species. Studying the toxicity of organic compounds of tin or eggs and larvae of several bony fish and cartilagenous fish (sturgeons), P.O. Danilchenko (1975), on the example of triethyl tin chloride, showed that embryonal development occurs in bony fish in solutions of this substance 10 times greater; in sturgeons, 100 times greater than the concentration in which prelarvae survive.

The decreased penetration of the shell for most organic poisons does not of course mean that they do not penetrate into the perivitelline fluid at all and do not reach the embryo. Organic chlorine pesticides, for example, have been found in the eggs (Dethlefsen 1975), but they are apparently adsorbed on the surface of the egg and only cases of high concentration and permeability disorders of the shell have a toxic effect on the embryo.

The increased sensitivity of eggs to toxins of various natures, as well as the difficulties arising in interpretation of experimental data obtained in experiments on eggs, lead to the need to use other substrates as test data in ichthyotoxicologic studies in evaluating the level of resistance of fish in the early stages of ontogenesis. Prelarvae, larvae and, particularly, fish fry which, like mature individuals (after the transition to gill breathing), have direct contact with the toxic agents, i.e., are under conditions comparable to those in which experiments are performed on mature fish, have doubtless advantages. Therefore, from the practical standpoint, our primary emphasis must be on data characterizing the dynamics of toxicoresistance of fish in the larval and fry periods of life, both to organic and inorganic poisons.

In the first part of our report, we analyzed the age specifics of the resistance of bony fish and cartilagenous fish in the larval and fry stage of life, using the model of phenol intoxication of fish performed in our laboratory. The fact of gradually decreasing resistance from larvae to fry and from fry to immature individual, we found has been repeated by many researchers in experiments with other organic poisons, including pesticides and detergents.

In contrast to organic poisons, toxic substances of inorganic nature and, in particular, heavy metal salts, are most toxic for fish "in the larval and fry stages" (Stroganov and Pazhitkov 1941). However, what are the dynamics of toxicoresistance of fish in the larval and fry periods of life, i.e., in the early stages of ontogenesis, we do not know due to the sparse nature of studies of this problem. D. Blaxter (1975) considers, for example, that the "sensitivity" of plaice larvae (meaning decreased resistance) increases with age. If "young" larvae survive in 1000 μg Cu/liter, 32-42 day larvae died at a concentration as low as 300 μg Cu/liter. G. Larson, et al. (1977) studies the acute toxicity of inorganic chloramino compounds for larvae with the yellow sac, fry and juvenile American brook trout (Salvelinus fontinalis). The fry were less resistance than the larvae and the lethal concentration (LC_{50}) of inorganic chloramines at 96 hours exposure for them was 82 μg/liter, for larvae with the yellow sac - 90-105 μg/liter. In the larvae, a decrease was noted in the resistance with increase in body weight.

In our laboratory in the last three years, we have performed a cycle of studies involving students from the ARE - Abbas Said Abu El-Ess, and from Iraq - Talyal Al Kubeysi and Adnan Musa Edzhad - on the age dynamics of toxicoresistance of larvae and fry of sturgeons with respect to common metals, cadmium and cobalt.

The experiments were performed on 1, 5, 10, 20 and 30-day-old larvae, as well as 40, 60, 90 and 120-day-old fry of the giant sturgeon, Russian sturgeon and Caspian sturgeon. We used the following concentrations of salts: cadmium chloride - 0.01, 0.1, 0.5, 1, 2, 4, 5, 8 and 10 mg/liter; cobalt chloride - 0.1, 1, 4, 5, 8, 10, 16, 32 and 64 mg/liter. The indication of resistance of the larvae and fry was the percentage of deaths and the time of survival in a solution of a given concentration of toxic substance. The duration of the experiments was 48 hours; observations were performed around the clock.

Summarizing the results of many series of experiments in this cycle, we conclude that the level of toxicoresistance of larvae and fry of these sturgeons differs significantly and that the larvae are significantly less resistant in comparison to the fry. However, within each of these two age groups of early ontogenesis, there is a significant change in toicoresistance, as indicated by the percentage and time of death of fish at the same concentration, as well as the threshold lethal concentration. For example, the toxicoresistance of the Russian sturgeon gradually decreases from the early stages of larval development to later stages, becoming minimal in the transition period (from larval to fry), then increases once more from the early age group to the later age groups, reaching a rather high level by the

60th day of age. Whereas, in the fry period of life in all three species we see the same direction of change of toxicoresistance (an increase from younger age to older age), in the larval period of life we see species specificity of the dynamics of toxicoresistance. In the giant sturgeon, the 10-day-old larvae were least resistant; in the Caspian sturgeon, the 20-day-old larvae; in the Russian sturgeon, the 30-day-old larvae.

Among the three species of sturgeons studied, the larvae of the giant sturgeon were least resistant to the salts of heavy metals, the larvae of the Caspian sturgeon were most resistant. The larvae of the Russian sturgeon occupied an intermediate position. The species specificities of toxicoresistance, which we observed, were manifested for each of the three indexes, lethal concentration, percent death and time of survival of experimental larvae in toxic solutions. For example, the lethal concentrations of cadmium chloride for larvae of the giant sturgeon of various ages were 0.1-1 mg/liter (LC_{50} = 0.5 mg/liter); cobalt chloride, 0.1-10 mg/liter (LC_{50} 10 mg/liter). A change in concentration of cadmium chloride by a factor of 100 had practically no influence on the level of toxicoresistance of the giant sturgeon in early ontogenesis, and the mean time of survival did not undergo significant changes in any of the three age groups of larvae. This is also fully true of the level of resistance of various age groups of larvae of the giant sturgeon in relationship to cobalt, although its toxicity is about 1/10 the toxicity of cadmium chloride.

The lethal concentration of cadmium chloride (LC_{100}) for Russian sturgeon (4 mg/liter) was 1/2 that for the giant sturgeon (8 mg/liter). The elevated resistance of Caspian sturgeon larvae, in comparison to Russian sturgeon, was also found in experiments with cobalt chloride, lethal concentrations of which were 64 and 32 mg/liter, respectively.

Age variability and the level of toxicoresistance in the early stages of ontogenesis are determined primarily by the degree of formation of various functional systems, to a lesser extent by changes in size (mass) of the body. A change in body mass by a factor of 4 for 10-120 day old fry (from 3 to 12 g) does not lead to any significant increase in the survival time of the fry of Russian sturgeon in toxic solutions of the metals studies.

As we know, cadmium is a highly toxic metal. Suffice it to say that the lethal concentrations of this metal for many species of fresh-water and marine fish fall in the range of 0.01-2 mg/liter (Lukyanenko 1976; Patin 1977). However, according to our data, a concentration of cadmium chloride of 4 mg/liter leads to the death of 10-day-old Russian sturgeon larvae in 14.6 hours; of 20-day-old larvae in 29.7 hours; 30-day-old larvae in 8.5 hours; while 60-day-old fry survive for 48 hours. Furthermore, 4-month-old fry survive in a solution of cadmium chloride of 8 mg/liter for 48 hours (only 105 of the experimental animals die). All of these data indicate that the cartilagenous fish, in this case Russian sturgeon, are significantly more resistant to the toxic effect of cadmium in comparison to marine and fresh-water species of bony fish in the early stages of ontogenesis.

Summing up our report on the age specifics of the sensitity and resistance of fish to poisons, I would like to draw the attention of participants in the symposium to still another very important, in my opinion, question. I am speaking of the great need for a clear delineation between the concepts of "sensitivity" and "resistance" of fish to poisons, which are quite different in their physiologic and toxicologic significance (Lukyanenko 1967). Unfortunately, quite frequently in both domestic and foreign literature, the concept of sensitivity and that of resistance of hydrobionts to various factors in the aquatic environment, as well as toxins, are either identified or sensitivity is considered to be the reverse of resistance. The use of these concepts as synonyms can lead and does lead to negative results, including difficulty in understanding the degree of scientific foundation of the conclusion of various authors who have estimated the age differences of toxicoresistance of fish.

There is a generally agreed idea, concerning the meaning of the concept of resistance of an organism to abiotic factors in the environment, concerning toxins of various natures. An estimate of the degree of resistance is based either on the concentration of the substance causing death of a certain percentage of experimental animals (LC_{50} or LC_{100}) in a certain period of time (24-48-96 hours or more), or the time of survival in a toxic solution of a predetermined concentration. Resistance is the capacity to survive low concentrations of a toxic substance for longer periods of time, or to survive higher concentrations of the same substance for a fixed short period of time by the operation of various regulatory mechanisms. Quite understandably, the earlier these regulatory mechanisms are brought into play (detoxication, excretion of the substance, etc.), supporting short-term or long-term adaptation of the organism to the toxic agent, the longer will be the time of survival of the organism and the more probable that, in the case of interruption of the toxic effect on the organism, it will survive. However, it is also obvious that regulatory mechanisms will be brought into play earlier, the more sensitive the organism is to the toxin at the given stage of individual development.

In terms of their physiologic content, the concept of "sensitivity" is close to or coincides with the concept of "excitability", the level of which determines the threshold of excitability. In turn, a measure of excitability is the minimum force of an irritant; in this case a chemical factor, which exceeds the threshold of irritation. The greater the minimum force of the chemical irritant necessary to call forth a reaction, the higher the threshold of irritation, the lower the excitability, the lower the sensitivity of the organism to the substance in question. Quite understandably, the lower the threshold of irritation, the higher the excitablity, and the higher the sensitivity. This is a generally known physiologic truth, in light of which we must analyze the question of sensitivity of the organism or cell to a toxic irritant. It follows from all of this that, in order to estimate the level of sensitivity of the organism to a given toxin, the question of the primary reaction of the organism to this irritant is of primary significance. I propose that there is no need to prove that neither the concentration of the substance causing the death of a certain percentage of experimental fish, nor the time of survival of fish at a fixed concentration, can be used in any way as an indication of the primary reaction to a chemical irritant. It becomes obvious from this that the widespread concept of sensitivity of fish to a poison as the "inverse of resistance" is without foundation.

We turned our attention to this inconsistency more than 10 years ago (Lukyanenko 1967) in our study of specific peculiarities of the toxicoresistance of mature fish to poisons on the model of phenol intoxication. Using rapid motor activity as an indication of the primary reaction of mature fish to the phenol irritant, its latent period, and the time of survival of the experimental fish as an indication of stability, we proved (Lukyanenko and Flerov 1965) that high sensitivity of a species is not always accompanied by low resistance and vice-versa. Of course, our concept of the degree of sensitivity of fish to various toxins will change depending on which functional system is selected as the indication of primary reaction. Everything is determined by the understanding of the mechanism of action of the toxic substance being studied, and the precise knowledge of the "functional target", since only using this function can we adequately determine the level of sensitivity. It is difficult to determine the target function, even in mature fish, to say nothing of the early stages of ontogenetic development and especially embryonal development. In the embryonal period, a toxic substance which penetrates the shell in many cases has its harmful influence not on organs and functions as such, but rather on processes determining the development of organs or the genesis of functions. If we agree with the current opinion (Bocharov 1975) that the sensitivity of the developing organism varies in various portions of the embryo, the task of evaluating the sensitivity of the embryo as a whole becomes still more difficult and responsible.

However, in many works dedicated to the toxicology of embryonal or larval stages of development of fish, the concept of "sensitivity" is used quite broadly and most frequently as the reverse of resistance. Therefore, the decreasing stability of developing larvae to a toxin is taken as evidence of increased sensitivity in comparison to mature, fully formed individuals of the same species. If we agree with this point of view, we must say that the organism of the fish as it develops, accompanied by formation of organs and development of functions, including the receptor function of the peripheral nervous system, somehow loses its sensitvity to chemical irritants (in this case toxins) in comparison to the developing embryo. From the physiologic standpoint, this interpretation of the change in sensitivity of the organism in onotogenesis is hardly acceptable. The developing egg contacts the surrounding medium and, consequently, receives external irritants with its entire surface. If a chemical substance which has toxic properties penetrates through the shell, its reception may be performed by the plasmatic membrane of the cells of the developing embryo, the ancient function of which is the reception of stimuli. However, it is hardly possible that the sensitivity, i.e., excitability of these cells, which are simple acceptor-receptor systems, could be higher than that of the specialized nervous system of a complex multicell organism such as a mature fish, responsible for the function of reception, conduct and acceptance of stimuli of physical or chemical nature.

We propose that in describing the reactions of fish to toxic irritants in the embryonal and immediate postembryonal periods of development (prelarval and larval), the concept of resistance be universally used. Sensitivity or susceptibility can be spoken of only if it is specially studied using adequate methods of investigation.

Returning to the primary point of the present report, I would like to emphasize that over the past decade, new data have been obtained, indicating the presence of clear age specifics in the sensitivity of fish to poisons. However, the level of toxicoresistance is determined not only by the direction and intensity of metabolic processes of fish in various stages of ontogenesis, but also by the nature of the toxic agent used. The resistance of various species of fish to many organic poisons decreases with ontogenetic development and reaches a minimum in sexually mature fish. However, this process is not uniform and periods of high resistance (egg in stage of pulsating heart, larva in C_2 stage and current year's brood) alternate with periods of low resistance (egg in stage of gastrulation, larva at end of larval period, immature individuals). Particular attention should be given to the end of the larval and the beginning of the fry period of development, when the resistance of fish to organic poisons drops sharply. As concerns the resistance of fish to inorganic poisons and, in particular, to heavy metal salts, it is minimal in the larval and fry period of individual development. The resistance of the fry (embryonal period of development), both to organic and to inorganic poisons, is significantly higher in comparison to the larval and fry periods. The nature of the increased toxicoresistance of the egg remains unclear. This factor makes the use of eggs as test objects (reference objects) undesirable in studies of the degree of toxicity of various substances for various stages of the ontogenesis of fish and biologic testing of natural and waste waters (larvae and fry are preferable).

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SECTION 13

SYNERGISTIC EFFECTS OF PHOSPHORUS AND HEAVY METAL LOADINGS ON GREAT LAKES PHYTOPLANKTON

E.F. Stoermer, L Sicko-Goad and D. Lazinsky[1]

INTRODUCTION

The Laurentian Great Lakes are one of the major physiographic features of North America. They represent a tremendous resource to the people of Canada and the United States. They provided European colonizers a route of access to the interior of the continent and continue to provide an important transportation artery, particularly for the raw materials of heavy industry. In the early decades of the present century the Great Lakes supported an important fishing industry and their waters furnished a seemingly inexhaustible supply of high quality potable water and industrial process and cooling water. As a result of these favorable circumstances the shores of the Great Lakes were a favored site for early settlement and have supported the growth of several major population and industrial centers.

Unfortunately, the byproducts of these populations and industrial concentrations have had effects on the Great Lakes ecosystem which damage the very resource potential which allowed their growth and development. During the past several decades important fish stocks have been severely damaged or, in some cases, entirely lost. Some of the stocks remaining have been contaminated by heavy metals or organics to the point that there are serious questions regarding their suitability for human consumption. Eutrophication has also caused modifications in the composition and abundance of primary producer communities which have had direct effects on the utility of Great Lakes waters. Overproduction and changes in composition of the phytoplankton assemblages of the Great Lakes have led to taste and odor problems in municipal water supplies and additional treatment costs for removal of biological materials from the water. Extreme overproductivity of benthic communities has resulted in nuisance growths of attached algae such as *Cladophora*.

These problems have been recognized and considerable effort has been directed towards defining the causes of water quality and associated re-

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source deterioration and implementing management strategies which will control or eliminate the particular problems. In many cases management strategies are clearly evident and considerable success has been obtained by their implementation. Perhaps the clearest case of success is the restriction of use of certain chlorinated hydrocarbon pesticides which has reduced the contamination levels of Great Lakes fish. In the Great Lakes system primary productivity is clearly controlled by phosphorus availability and efforts are underway to limit inputs of this material to the system. This limitation has proven more difficult to implement and positive effects, to this point, have not been dramatic.

As we become more familiar with the characteristics of the Great Lakes ecosystem it becomes more and more apparent that effective management will demand a detailed understanding of ecosystem characteristics and functional relationships in order to develop management strategies which can control subtle and multiplicative causes of ecosystem deterioration. Consideration of the unique characteristics of the Laurentian Great Lakes leads to the conclusion that these bodies of water may present the most demanding challenge to effective water quality management found in any freshwater system. Several considerations are involved in this conclusion:

1. In their pristine state the Laurentian Great Lakes were an almost perfectly exploitable system. They were a source of water which could be utilized without extensive treatment and supported a fishery for very highly valuable species. They were also a source of aesthetic enjoyment and recreational activities for a significant portion of the population. Minimal levels of perturbation led to disproportionately large damage to the resource potential compared to other systems.

2. The Great Lakes are a geologically very young ecosystem, compared to most large lakes of the world. The fauna and flora are unique but have not had time to develop stable adaptations to their environment. Such communities might be expected to be particularly susceptible to environmental perturbation and this expectation has been realized in the history of biological changes observed.

3. The Great Lakes are very long residence-time systems compared to most other freshwater biotopes. This means that introduced contaminants may have very prolonged effects.

4. Because of the great dilution volume of the Great Lakes contaminants may be present in quantities so low that they are difficult to measure by conventional chemical methods although their effects may be crucial to the biota.

5. It is quite clear that the classification and perception of water quality developed for other freshwater systems is not appropriate for the Great Lakes. Paradoxically, drastic and possibly irreversible modifications of the Great Lakes eco-

system have occurred in regions that would be classified as "oligotrophic" according to the normal criteria.

In the following report we will attempt to address some of the interactive effects of two types of contaminant loadings, phosphorus and heavy metals, which might not be discerned by conventional limnological methods. The research was originally initiated in an attempt to explain the apparent differential influence of phosphorus enrichment on particular species of phytoplankton advected through zones of phosphorus pollution. Loadings, biological availability, and biological pathways of this nutrient in the Great Lakes system are of particular interest because it is the primary nutrient controlling eutrophication. Most undesirable anthropogenic modifications of the Great Lakes ecosystem are directly related to increased phosphorus loadings resulting from increased population densities, introduction and widespread usage of phosphorus containing detergents, and poor land management practices. In the course of this investigation we found that the mechanism allowing differential sequestering of phosphorus was intimately associated with heavy metal concentration in the water and that the same mechanism could permit excessive uptake of certain toxic metals. Since this bioaccumulation mechanism could have both effects on the aquatic ecosystem and potential effects on human health we have attempted to determine some of the factors involved.

Since the problem we are dealing with has not, to our knowledge, been previously investigated in the context of large lake limnology and since some of the methods we have adopted have not been widely employed in water quality investigations it would perhaps be helpful to give a brief chronological outline of the development of this investigation before discussing results.

During an investigation of Saginaw Bay, one of the more grossly polluted regions within the Great Lakes ecosystem, it became apparent that certain species of phytoplankton were surviving transport out of the bay into Lake Huron. This was unexpected because the species involved have high nutrient requirements which cannot be satisfied in Lake Huron. We hypothesized that populations within the bay were taking up phosphorus in gross excess of their immediate physiological requirements and subsequently surviving transport out of the nutrient-rich environment by using these internal stores. In order to verify this hypothesis we examined the internal cellular constituents of these populations by analytical electron microscopy. This analysis confirmed the presence of internal stores of phosphorus in the form of polyphosphate bodies. X-ray analysis further showed that the polyphosphate bodies also contained appreciable quantities of lead. Subsequent field observations in areas subjected to combined phosphorus enrichment and heavy metal contamination indicate that the phenomenon observed in Saginaw Bay is common in other parts of the Great Lakes system. Laboratory studies were also carried out to determine if other metals behave in the same manner as Pb.

MATERIALS AND METHODS

The observations reported here come from natural phytoplankton assemblages collected and fixed under field conditions, natural assemblages brought into the laboratory and subjected to experimental nutrient and heavy metal additions, and populations isolated from the lakes and maintained in the laboratory.

Culture Conditions

Natural assemblages used for experiments were returned to the laboratory within 5 hours of collection in 20-ℓ prerinsed plastic containers. Containers were placed in an insulated, light-tight box for transport to avoid temperature and light shock. In the laboratory experimental material was maintained in a culture chamber at the temperature of collection (± 1.0°C), and 200 μ Ein m^{-2} sec^{-1} of illumination on an alternating 16-hr day, 8-hr night cycle.

Cultured material was grown in FM medium (Lin and Schelske 1978) at 15°C at the same illumination and daylength conditions used for natural assemblages.

Light Microscopy

All observations reported were made with a Leitz Ortholux microscope with immersion objectives furnishing numerical aperature of at least 1.30. Cells were stained for polyphosphates by the method of Ebel *et al*. (1958) and were observed and photographed either in temporary aqueous mounts or in permanent mounts embedded in Epon prepared by the same method used for electron microscopy. Photographs were taken with a Leitz Orthomat photo apparatus.

Electron Microscopy

Material was fixed with 3% (vol./vol.) biological grade glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) for one hour at 4°C and post-fixed in 1% OsO_4 for 1 hour. Cells were dehydrated in a graded ethanol-propylene oxide series and embedded in Epon (Luft 1961).

Thin sections were cut with a diamond knife, collected on 300 mesh grids and stained with uranyl acetate (Stempak and Ward 1964). Sections were examined on a Zeiss EM 9S-2 electron microscope. Microscope magnification calibrations were made by use of a grating replica.

X-Ray Analysis

Sections for X-ray analysis approximately 60 nm thick were cut with a diamond knife and collected on 75X300 mesh titanium grids. Sections were examined at 100 KV in STEM mode in a JEM 100C electron microscope equipped with a KEVEX series 7000 energy dispersive X-ray analysis system. The specimen was tilted 30° toward the detector. Specimen to detector distance was 18 mm. Spot analysis of inclusions was made with a spot size of 50 Å.

Stereology

Quantitative estimates of cellular components were developed by techniques described by Sicko-Goad et al. (1977). Fifty micrographs were examined for each experimental treatment analyzed. A transparent 12.5 mm square sampling lattice was superimposed over the micrographs for point count measurements. Although several sections were collected on one grid, only one section per grid was used in the analysis. Blocks were retrimmed after each series of sections had been cut in order to avoid repeated sampling of adjacent material within the same organism. For species where cells are connected in a colony, only one cell per colony was included in the statistical sample.

RESULTS

Figure 1 shows the distribution of Fragilaria capucina Desm. in southern Lake Huron in June of 1974. This distribution is atypical in that this species generally becomes abundant in areas of the Laurentian Great Lakes which are severly eutrophied (Hohn 1969) but does not survive in the less nutrient rich offshore waters. Electron micrographs of cells of this species taken within Saginaw Bay (Figure 2) show that they contain numerous small vacuolar inclusions having the general form and appearance of polyphosphate bodies. Although the formation of polyphosphate bodies has not been widely reported in eukaryotic phytoplankton organisms, X-ray analyses of the inclusions (Figure 3) confirm that their elemental composition is essentially similar to that of polyphosphate bodies reported from prokaryotic organisms (Sicko-Goad et al. 1975). The primary difference is that the bodies found in Fragilaria capucina are much smaller than those found in most prokaryotic organisms and that they are found within the vacuole of the eukaryotic cells.

X-ray spectra of the polyphosphate bodies found in Fragilaria capucina in this locality also indicate the presence of appreciable quantities of Pb as a constituent of the bodies (Figure 3).

Observations of other eutrophication tolerant phytoplankton species in Saginaw Bay indicated the widespread occurrence of polyphosphate bodies, even in areas where chemical analyses of the water showed low levels of dissolved phosphorus in the water. Polyphosphate bodies were particularly apparent in cells of some of the potentially nuisance producing blue-green algae in the assemblages. These observations also show that the distribution of populations containing polyphosphate bodies within the bay is restricted primarily to stations along the southern and southwestern shore of the bay (Figure 4).

Subsequent observations utilizing staining techniques which permit visualization of polyphosphate bodies at the light microscope level (Ebel et al. 1958) show that polyphosphate bodies are developed in phytoplankton populations present in several areas of the Great Lakes system which receive relatively high loadings of phosphorus and other contaminants.

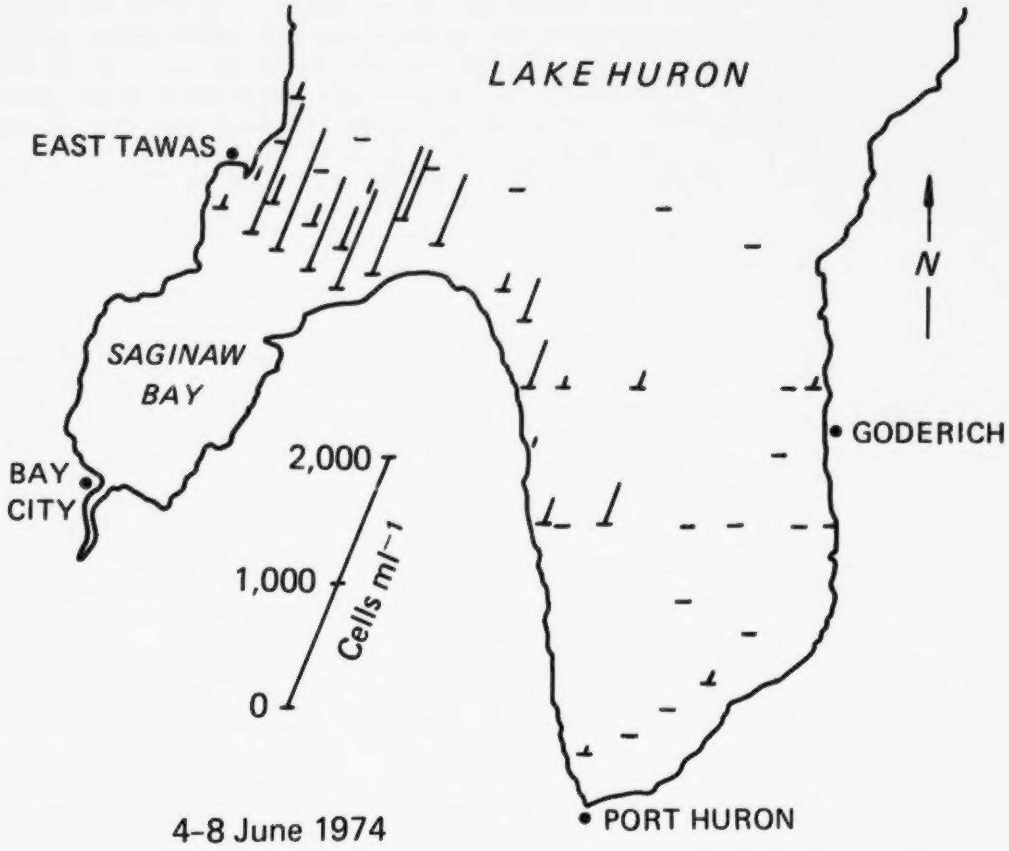
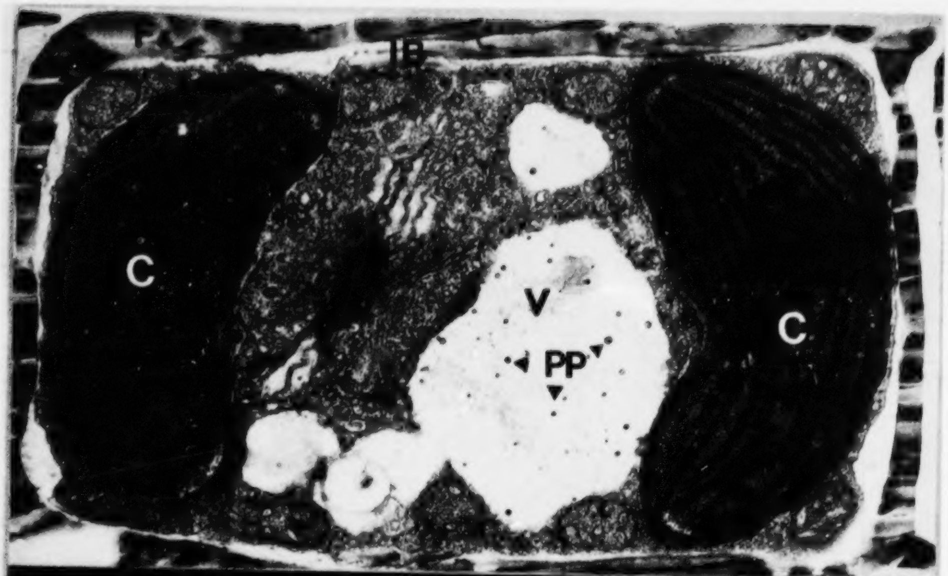

LAKE HURON
EAST TAWAS
SAGINAW BAY
BAY CITY
GODERICH
PORT HURON
N
2,000
1,000
0
Cells ml⁻¹
4-8 June 1974

Figure 1. Outline map of the southern Lake Huron showing the distribution of the eutrophication tolerant diatom Fragilaria capucina Desm. in the waters of Lake Huron outside Saginaw Bay in early June 1974.



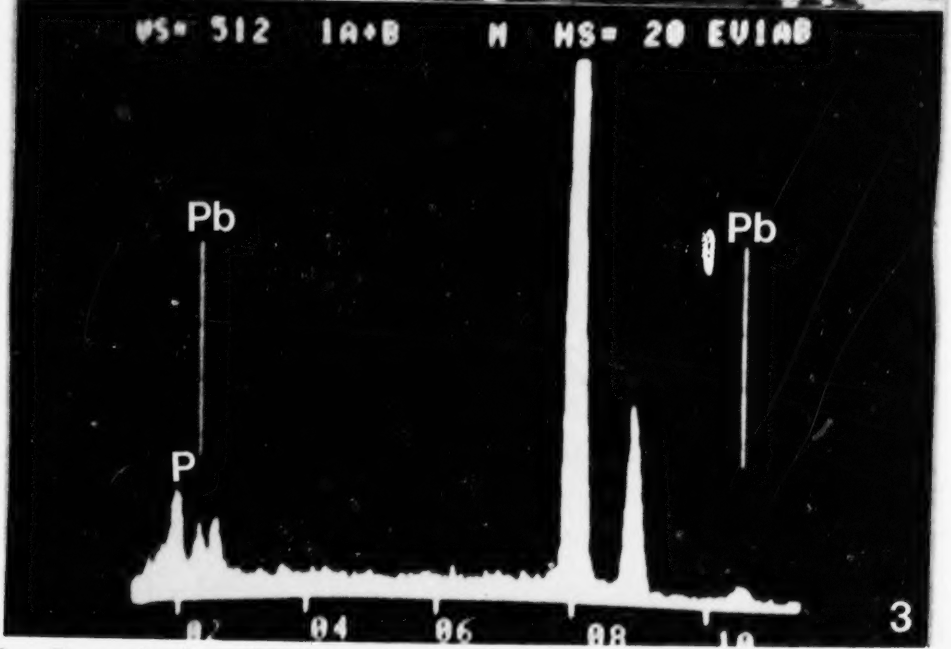


Figure 2. Transmission electron micrograph of a cross section of Fragilaria capucina. Numerous small polyphosphate bodies (PP) are present in the vacuole (V). Other cytoplasmic organelles are normal. Large chloroplasts (c) are positioned under the valve face of the frustule (F). Golgi apparatus (G) appears somewhat disorganized because the intercalary bands (B) are being formed prior to next cell division. (Magnification X29,000).

Figure 3. X-ray spectrum of a polyphosphate body contained in the vacuole of Fragilaria capucina. The labelled peaks are P (Kα) and Pb (Mα, Lα). A minor calcium peak (Kα 3.69 Kev) is also present. Unlabelled peaks are Cl (Kα 2.62 Kev), a component of the epoxy embedding medium, and Cu (Kα 8.04, 8.02 Kev; Kβ 8.90 Kev), which originates from the grid.

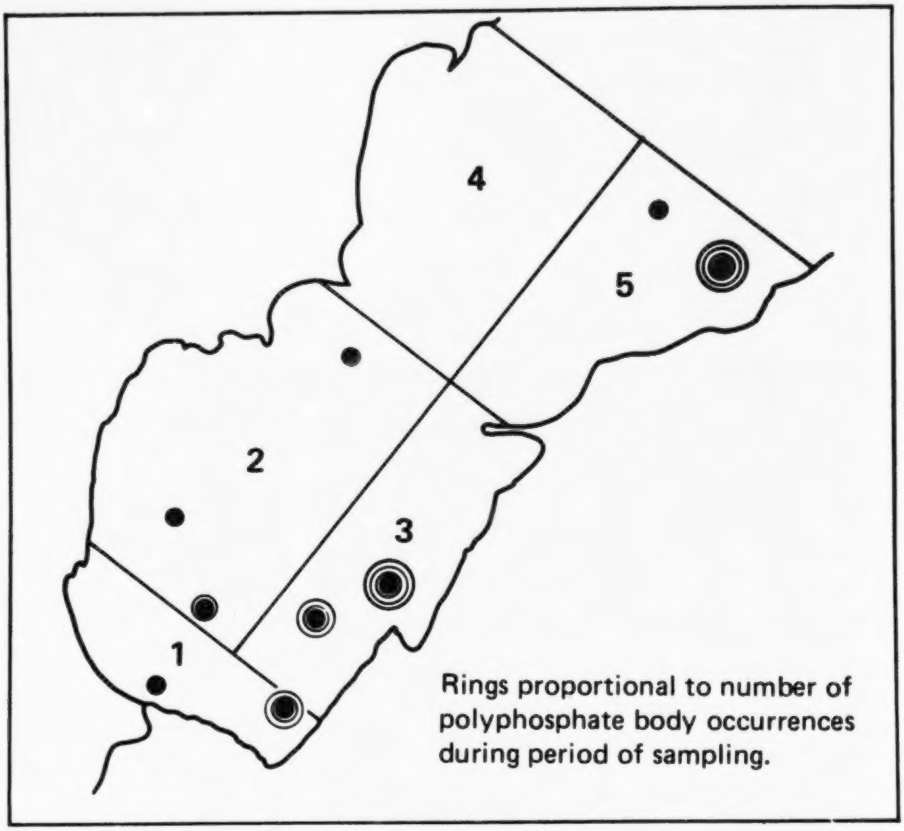

1
2
3
4
5
Rings proportional to number of polyphosphate body occurrences during period of sampling.

Figure 4. Outline map of Saginaw Bay, Lake Huron showing the abundance of algal populations containing polyphosphate bodies in different segments of the bay (Smith *et al.* 1977). Average circulation is counterclockwise and polyphosphate bodies are most common downstream of the Saginaw River pollution source.

The form and position of these inclusions is somewhat different in the various major physiological groups of phytoplankton. Polyphosphate bodies in the blue-green algae may become large compared to the volume of the cell within which they are contained and their position within the cell is highly variable (Figure 5). In the green algae, as in most other eukaryotic cells, polyphosphate bodies are restricted mainly to the vacuole. In the species we have examined so far, there is considerable variation in the relative size and position of the bodies present (Figures 6 and 7).

In diatoms polyphosphate bodies are usually very small (< 0.5 μm) (Figure 2) and are usually positioned near the vacuolar membrane inside the vacuole, although they may become dispersed in the vacuole (Figures 2 and 8).

Among the flagellate groups, polyphosphate bodies similar to those found in diatoms have been noted in various members of the Chrysophyceae (*sens. str.*) and the Prymnesiophyceae. Interestingly, they seem not to be present in the Cryptophyceae and we have not found them in Euglenoids, although our samples of these organisms are small, since they are very rare in the Great Lakes.

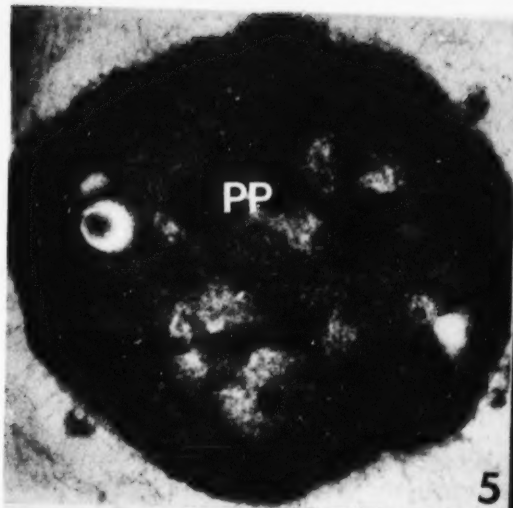
Since we had observed accumulation of Pb, but not other metals in field samples, we decided to test for possible differential uptake of different metals under controlled conditions. The metals tested were Pb and copper, which is known to be rather acutely toxic to many species of algae (Fitzgerald and Faust 1963). A unialgal culture of *Diatoma tenue* var. *elongatum* Lyngb., originally isolated from Lake Michigan was grown in FM medium. Since phosphorus limitation followed by phosphorus excess is one of the conditions known to initiate polyphosphate body formation (Jensen and Sicko 1974) phosphorus starvation and phosphorus excess were simulated in the following manner. Four-day-old cultures which were in logarithmic growth (controls) were packed by gentle centrifugation, washed twice with sterile distilled water, then inoculated into a medium of the same composition of FM medium except that it lacked phosphate salts. Cells were incubated in this medium for 3 days to induce phosphorus starvation. At the end of the starvation period, during the fourth hour of the culture light cycle, cells were again packed by centrifugation and resuspended in one of the 3 following media as treatments:

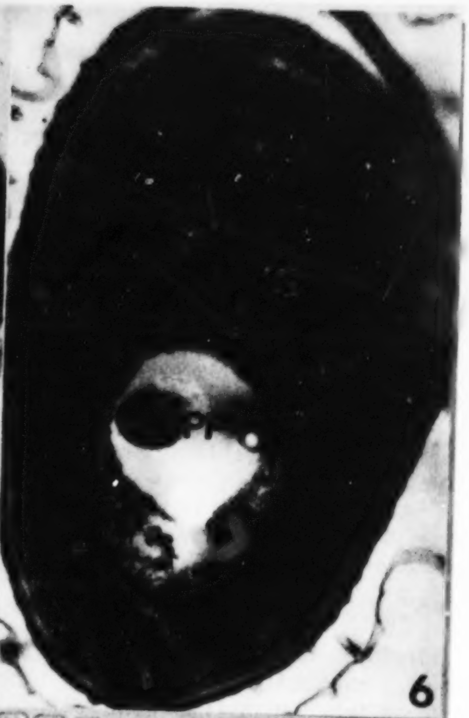
1. Medium containing twice the phosphorus concentration of FM medium with no other additions.

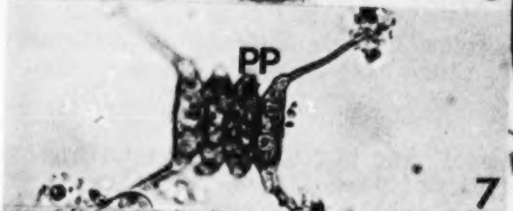
2. Medium containing twice the phosphorus concentration of FM medium + 0.05 μg-at/ℓ Pb.

3. Medium containing twice the phosphorus concentration of FM medium + 0.08 μg-at/ℓ Cu.

Cells were incubated under normal culture conditions in these treatments for 2 hours then fixed and prepared for electron microscopy along with control samples. Splits of the samples were also stained for polyphosphates and prepared for observation under the light microscope.







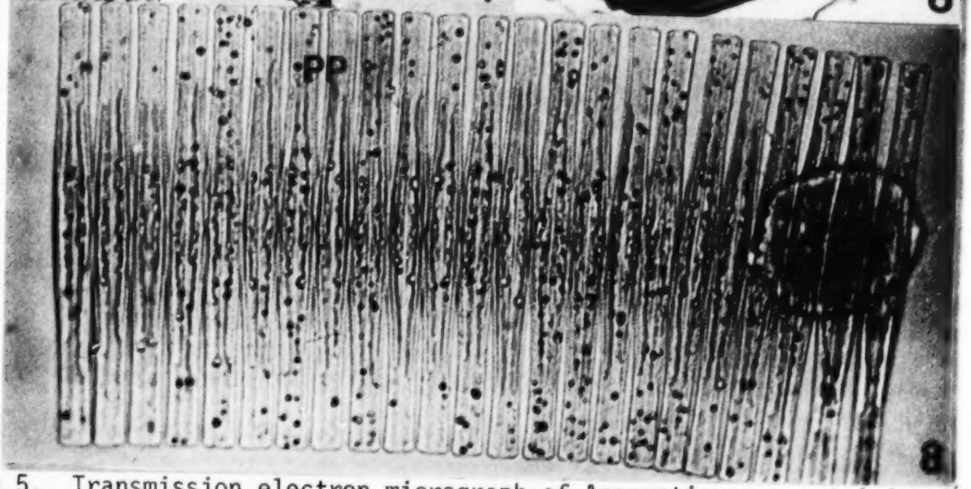


Figure 5. Transmission electron micrograph of *Anacystis* sp. containing large polyphosphates bodies (PP). (X53,000).

Figure 6. Transmission electron micrograph of *Scenedesmus* sp. showing large polyphosphate bodies (PP) in the vacuole. (X23,000).

Figure 7. Light micrograph of *Scenedesmus* sp. stained for polyphosphates by the technique of Ebel *et al*. (1958). Material is from a natural phytoplantkon assemblage enriched with phosphorus and heavy metals. (X1,700).

Figure 8. Light micrograph of *Fragilaria crotonensis* Kitton stained for polyphosphates by the technique of Ebel *et al*. (1958). Material is from a natural phytoplankton assemblage enriched with phosphorus and heavy metals. (X800).

Electron micrographs of sectioned material from control cultures and all treatments were analyzed by stereology to quantify polyphosphate body abundance under the conditions tested and to determine other changes in cellular structure which might be induced by the treatments. Sectioned material was also subjected to X-ray analysis to verify polyphosphate body composition and metal accumulation. The results of this analysis is given in Table 1.

Preliminary results from work currently in progress indicates that heavy metal stress results in increased polyphosphate body formation in *Plectonema boryanum* Gom. (Figures 9-11). These results further indicate a differential effect depending on the degree of direct toxicity of the metal to the alga subjected to the stress. In *Plectonema* Pb and zinc cause an approximately 10-fold increase in polyphosphate bodies per cell after 3 days exposure. Copper and cadmium treatments result in a ca. 5-fold increase, but increased apparent cellular damage at the ultrastructural level.

DISCUSSION

Our results are indicative of the complex and poorly understood cellular level interactions which may occur in algal populations of large lakes subjected to nutrient and toxicant contamination. Previous reports in the literature suggest polyphosphate accumulation may be triggered by several types of nutrient imbalance (see Sicko 1974 for review). It is important to note that the mechanism may be triggered either by deficiency in some critical nutrient in the presence of excess exogenous phosphorus (Lawry and Jensen 1979), stress invoked by excess levels of micronutrients, or simply by the restoration of excess exogenous phosphorus to cells previously stressed by deficiency of this nutrient.

Any or all of these conditions are apt to be present in mixing zones where contaminated stream flows enter the Laurentian Great Lakes. It is thus highly probable that rapid uptake of phosphorus in these areas is not directly related to the immediate growth potential of the algal populations affected. This is illustrated by our results from Saginaw Bay (Figure 4). The normal water circulation of the bay is counterclockwise with water exiting the bay along the southern shore (segments 3 and 5 in Figure 4) being replaced by Lake Huron water entering the bay along the northern coast (Danek and Saylor 1977). The primary source of nutrient enrichment and heavy metal contamination is the Saginaw River (Smith *et al*. 1977) which enters the far southwestern tip of the bay. In this case polyphosphate bodies are much more abundant in phytoplankton populations taken at stations downstream, in the sense of the average current vector, of the source than in other segments of the bay. It further appears that phosphorus bound in this form is transported out of the bay since polyphosphate bodies are found at stations near the mouth of the bay. The eventual fate of this material in the Lake Huron system cannot be determined on the basis of our observations. We would speculate, however, that at least two effects may occur. The first is that phosphorus bound in this form may eventually be reutilized allowing the survival of phytoplankton populations which are usually restricted to eutrophic areas in the open waters of Lake Huron. Other investigations (Stoermer and Kreis, in press) have shown that populations which appear to

TABLE 1. MORPHOMETRIC RESULTS OF NUTRIENT TREATMENTS.
RESULTS ARE THE MEAN ± 1 S.E.M.

	Control	PO Starved	PO Uptake	PO + Pb Uptake	PO + Cu Uptake
Frustule (V_v)[1]	17.8 ± 2.12	18.5 ± 1.66	18.0 ± 1.06	18.0 ± 1.34	17.7 ± 0.82
Chloroplast (V_v)	16.4 ± 1.08	15.5 ± 0.96	15.1 ± 0.67	15.5 ± 0.96	15.7 ± 0.94
Mitochondria (V_v)	3.07 ± 0.27	2.5 ± 0.27	2.7 ± 0.27	2.9 ± 0.27	3.0 ± 0.27
Mitochondria (N_v)[2,3]	$0.23/\mu m^3$	$0.21/\mu m^3$	$0.23/\mu m^3$	$0.17/\mu m^3$	$0.22/\mu m^3$
Ave. Vol.	$0.13 \mu m^3$	$0.12 \mu m^3$	$0.12 \mu m^3$	$0.17 \mu m^3$	$0.14 \mu m^3$
Number/Cell	64.4	52.5	50.6	37.4	50.6
Residual Bodies (V_v)	1.4 ± 0.25	2.0 ± 0.33	2.0 ± 0.28	2.1 ± 0.34	2.0 ± 0.25
Residual Bodies (N_v)[4]	$0.08/\mu m^3$	$0.09/\mu m^3$	$0.11/\mu m^3$	$0.22/\mu m^3$	$0.12/\mu m^3$
Ave. Vol.	$0.18 \mu m^3$	$0.22 \mu m^3$	$0.18 \mu m^3$	$0.10 \mu m^3$	$0.17 \mu m^3$
Number/Cell	22.4	22.5	24.2	48.4	27.6
Vacuole (V_v)	34.1 ± 1.68	42.8 ± 1.89	40.8 ± 1.56	42.1 ± 1.55	41.4 ± 1.47
Cytoplasm (V_v)	24.3 ± 1.34	18.8 ± 1.28	21.5 ± 1.32	19.3 ± 2.30	20.2 ± 1.09
Storage (V_v)	2.91 ± 0.58	0	0	0	0
Polyphosphate Bodies per μm^3 vacuole[5]	0.9	0.5	7.6	7.9	3.6

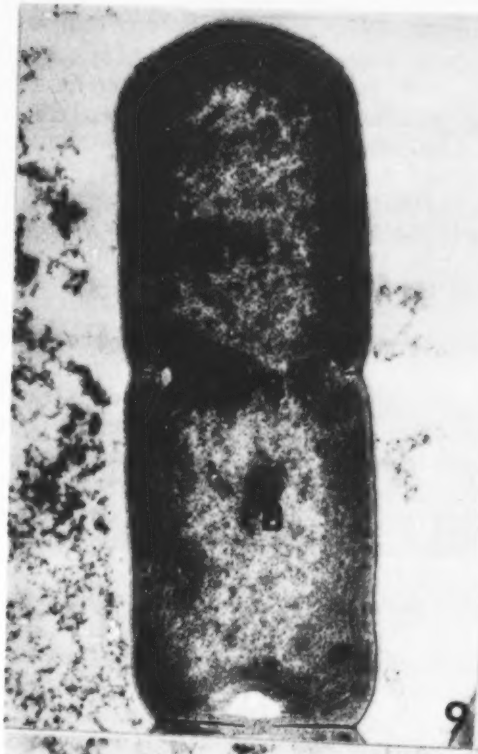
[1] V_v = relative volume.

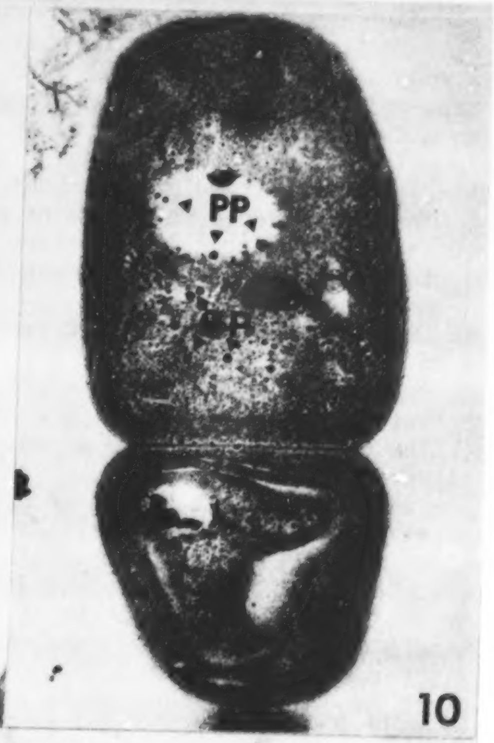
[2] N_v = number per volume.

[3] $N_v = \frac{K}{\beta} \frac{N_a{}^{3/2}}{V_v{}^{1/2}}$.

[4] $K = 1.07$, $\beta = 1.44$.

[5] Calculated by the formula $N_v = \frac{N_a}{\bar{D} - 2p + T}$.





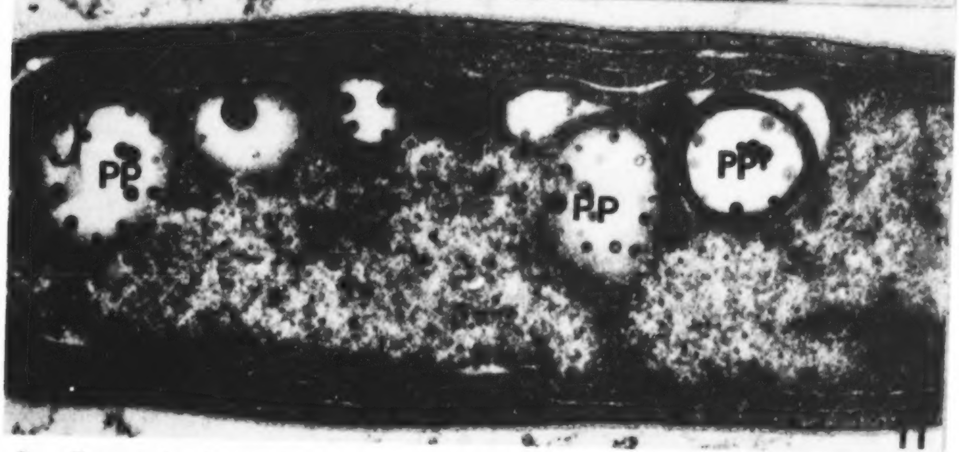


Figure 9. Transmission electron micrograph of cytologically normal *Plectonema boryanum*. Note regular cell septae (arrow) and polyhedral bodies (PB). (X28,000).

Figure 10. Transmission electron micrograph of *Plectonema boryanum* treated with 0.1 µg-at/ℓ Pb. Note increased vacuolization of the cell, apparent reduction in number of polyhedral bodies, and the presence of numerous polyphosphate bodies (PP). (X28,000).

Figure 11. Transmission electron micrograph of *Plectonema boryanum* treated with 0.1 µg-at/ℓ Zn. Note increased vacuolization of the cell, apparent reduction in the number of polyhedral bodies, numerous polyphosphate bodies (PP), and lack of cell division. (X45,000).

originate in Saginaw Bay under certain conditions can survive transport into the extreme southern part of Lake Huron. It is difficult to imagine this occurring unless these populations were growing fast enough to replace grazing and sinking losses. The other plausible effect is that death of these populations, through grazing or other process, will release additional phosphorus and thus stimulate eutrophication of the offshore waters of Lake Huron. To our knowledge this type of biological loading has not been considered in the limnological literature, but it may be an important mechanism of pollutant dispersal in the Laurentian Great Lakes.

Our data also suggest that incorporation of Pb in polyphosphate bodies may be an important mechanism for dispersal of the toxicant in aquatic systems. To our knowledge, our report of the polyphosphate-lead association is the first demonstration of this mechanism in naturally occurring populations. The fact that this type of uptake can be produced in the laboratory conditions and Crang and Jensen's (1975) demonstration of titanium incorporation in polyphosphate bodies in *Anacystis nidulans* Dr. and Daily suggests that binding of heavy metals in osmotically inert inclusions such as polyphosphate bodies could be a general mechanism for protecting phytoplankton cells (at least temporarily) against heavy metal toxicity. Our results to date suggest that this is probably not the case. Our experiments with metals more directly toxic to algae, such as Cu and Cd, as well as Zn show that although stress induced by the presence of these elements at relatively low levels may induce polyphosphate body formation, these elements are not sequestered in the polyphosphate bodies to any measurable extent. This situation should be further investigated as it is possible that organisms other than those so far investigated may be able to affect heavy metal incorporation in polyphosphate bodies or that incorporation may take place at concentrations other than those tested.

Our results are also interesting in respect to previous reports of heavy metal accumulation in algae. Silverberg (1975) demonstrated that Pb accumulated in the cell wall and in the peripheral vacuole of *Stigeoclonium tenue* (Ag.) Kutz. Silverberg (1976) also found that exposure of 3 species of green algae to relatively high levels of Cd resulted in degenerative changes in the mitochondria of the cells and the formation of granules within the mitochondria which apparently contained Cd. Although we have observed some changes in cellular organelle structure in our experiments, we have not observed measurable accumulation of Cd or Zn associated with any organelle of specific cellular site. It should be noted that the concentrations used in Silverberg's experiments were 3 to 10 times higher than the concentrations tested in our experiments. It is probable that the cellular modifications he noted are symptomatic of acute toxicity.

At this stage of our investigations many questions remain to be answered. We are, none the less, encouraged in that the application of modern instrumentation and techniques has provided some insight to the complex interactions of nutrient and heavy metal contamination in large aquatic systems. It is clear that an understanding of cellular level processes is essential to understanding system level processes and the development of effective management strategies. In the particular case of the Saginaw Bay pollution problem application of these techniques has elucidated a mechanism which would be exceedingly difficult to discover by conventional limnological methods.

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SECTION 14

REVERSIBILITY OF INTOXICATION AND FACTORS GOVERNING IT

I.V. Pomozovskaya[1]

Criteria characterizing the poor state of the aquatic environment and its inhabitants, their degradation and pathology when affected by various kinds of pollutants have been developed intensively during recent years. One of the industries with the largest water requirement is the pulp and paper industry. Wastes coming from this type of enterprise are among the most complicated and multi-factorial toxic complexes. In this connection, the attention given to the study of the effects exerted by wastes from these enterprises on bodies of water and aquatic organisms is quite natural.

Aquatic toxicological experimentation conducted in the zone of action of such mills have provided valuable data on the real danger of waste waters, the effects of their separate components, and their complexes upon aquatic organisms of varying organisation and taxonomic ranking. These studies have enabled a comparison of biological effects, related to the functioning of various waste treatment plants, and have provided recommendations for their most economic and rational reconstruction and exploitation.

In this type of work carried out for a few years in Karelia, the main criteria of toxicity chosen were the survival time of organisms, symptoms of intoxication, changes in growth development and reproduction (fecundity, quality of progeny, rate of maturation and spawning, etc), and alterations in indices of the functional state; such as gas exchange, hematology, and the degree and pattern of reversibility of intoxication.

The problem of reversibility of intoxication of organisms occupies a special position in the whole complex of methodical approaches. Intoxication of fish and other organisms is highly probable, even in the presence of a space limited point-sources pollution, since such sources may be on the direct route of migration of the organism.

An inquiry into the problem of the possible reversibility of intoxication may assist in predicting results for organisms that undergo short duration exposure in the polluted zone during crises, and in the case of salvo discharges. This index should be considered when the remote consequences of prolonged low-dose intoxication are in question, in assessing the degree of toxicity of one chemical reagent or another, and in determining the resistance of organisms to toxicants.

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Reversibility of intoxication implies the recovery of organisms to their normal physiological state after some pathological shifts brought about by a toxic agent. The reversibility of pathological processes is possible only at a definite concentration, and at a given duration of exposure to a toxic substance. It may be said that pharmacological practice is based on this phenomenon, since all pharmaceuticals employed are also toxins; but in a definite combination they are of use for the organisms. Such combinations, at which changes occurring under influence of poisons demonstrate reversibility, should also be understood in the area of aquatic toxicology.

Data from literature on this problem are fairly scanty and, in some instances, contradictory. Evidence of these facts can be found in the works by Jones (1947, 1951, and 1957), Schweiger (1957), Wuhrmann and Woker (1950), and Stroganov and Pozhitkov (1941), in which reversibility of intoxication in fish as affected by cyanides, sulphides, chloromercury, ethyl alcohol, salts of heavy metals, and phenols, has been investigated.

The dynamics of phenol intoxication reversibility have been described in a study by Lukyanenko and Fluorov (1963). Studies by Mann (1958), Ludemann (1962), Chernysheva (1968) and others have been concerned with reversibility of intoxication in fish as affected by insecticides. In these reports, the possibility of restoring the vital activity of fish which have been intoxicated with organophosphates is shown. Similarly, the irreversible phenomena arising from contact with organochlorine compounds is also demonstrated. A high degree of reversibility has been demonstrated under the influence of detergents (Libmann 1960), but the resistance of fish to various diseases decreases drastically.

This study has employed unpurified multi-component wastes from sulphate pulp production as toxicants in various modifications and dilutions. Further, sewage from sewage treatment plants has also been used. Waste waters utilized contained methyl mercaptans, sulphides, hydrosulphides, sulphates, acids and alkalis, methyl alcohol, furfurol, acetone, ammonia and other organic and mineral compounds. The water in the natural effluent receiver is similar in chemical composition to the average composition of wastes resulting directly from production. It is nearly oxygen-free and has a high carbon dioxide content (25.1 mg/ℓ). Different quantities of sulphur-containing compounds have been found in wastes from boiling and evaporating shops. They possess a strong hydrogen sulphide smell. These wastes contain alkali and some fairly toxic organic substances, including terpentine, methanol, acetic and other acids. Wastes from the heat-and-power stations are distinguished by a considerable amount of mechanical suspensions, the result of burning slurry lignin, bark, and fuel oil, and by their sulphur trioxide and sulphur dioxide content.

Atlantic salmon (*Salmo salar*), cisco (*Coregonus albula*), roach (*Rutilus rutilus*), perch (*Perca fluviatilis*) and pike (*Esox lucius*) were test species. Fish of the first year of life (from the moment of hatching until the transition to the fingerling stage) were used in contrast to fish of older age groups.

The species of the fish, its age, average weight, and state (motor activity, respiratory rhythm, pattern of food uptake, response to external stimuli, etc.) were determined before the experiment.

Fish were pre-adapted to laboratory conditions and were placed for a definite time in both concentrated and diluted waste water. When characteristic signs of intoxication appeared, these organisms were transferred to pure lake water where changes in their state, and the time and sequence of restoration of the functions lost were subsequently recorded.

The main sign of intoxication, which served as a signal for transferring fish to pure water, was most often the loss of the equilibrium reflex, and a transition to the inverted state. In some cases, the fish were subjected to a sequence of two to four exposures in the waste waters. The degree and dynamics of intoxication reversibility depended upon the temperature, the concentration of toxicants, the duration of exposure, the test species, and the age of the fish.

The maximum duration of the experiments was 30-35 days. Observations have shown that the resistance of organisms to toxicants depends on all of the factors noted above, but primarily upon the concentration of the agent, its chemical structure, and duration of exposure.

Symptoms of intoxication of similar types can be traced in the behavior of fish in test medium. The first phase of this phenomena involves increased excitability (violent movements, sometimes whirling, with increased respiratory activity). This phase is followed by a passive state (loss of the equilibrium reflex, lateral or inverted position, respiration depressed, refusal of food, loss of the shoaling effect, and changes in color. The degree, time, and pattern of manifestation of intoxication symptoms are also dependent on quite a number of factors. The most distinct, although brief, symptoms of intoxication are observed in concentrated media. In some cases these effects are obscure, especially in juveniles. In some phases they are entirely absent.

In this paper, attention was focused mainly on juvenile fish, since they inhabit the littoral part of a body of water which is most subject to contamination. Furthermore, special investigations have indicated that wastes issuing from sulphate pulp mills do not possess repellent properties for fish. Numerous experiments have demonstrated that brief contact with concentrated or weakly diluted wastes results in an irreversible intoxication of fish.

Thus, in 7-day-old larvae of Atlantic salmon (average weight 98 mg) kept in both undiluted and diluted (1:1, 1:1) waste, vigorous excitation was instantly recorded, coupled with serpentine movements and whirling activity. After six minutes, the larvae descended to the bottom in lateral position, failed to respond to stimuli, and their rate of respiration was diminished. After the larvae were transferred to pure water, restoration of normal breathing activity was observed after 15-20 minutes. They began to respond to external stimuli, and by the end of the first day of detoxification, the test larvae could not be distinguished from the controls by appearance alone. During the first day no deaths were observed. By the 7th day the larvae transferred from the undiluted wastes died. The dynamics of the survival rates for fish in pure water after intoxication are shown in Figure 1.

An approximately similar situation was observed when 37-day-old salmon larvae (mixed feeding stage) were exposed. The characteristic symptoms of intoxication were recorded after an exposure duration of four minutes. The whole complex of symptoms (strong excitation, persistent loss of equilibrium, and inverted position) was clearly seen in concentration wastes. In pure water, the fish died within the first day after exposure.

In dilutions 1:1 and 1:2, test organisms were very excited. When transferred to pure water, they retained this increased motor and respiratory activity for 30 minutes, subsequently sinking to the bottom of the tank and reacting to stimuli with only weak movements of the caudal fin. Food was refused and by the end of the third day of detoxification, the survival rate was only 10% (Table 1).

TABLE 1. REVERSIBILITY OF INTOXICATION CAUSED BY EFFLUENTS IN JUVENILE SALMON
(Age - 37 days, Mean weight - 144 mg, Temperature - 24°C, Exposure 4 minutes)

Dilution of toxicant	Condition of fish after exposure	Survival (%) in clean water		
		1 Day	2 Days	3 Days
Control	Active	100	100	80
1:2	Very active	20	20	10
1:1	Very excited	20	10	10
Undiluted waste	Equilibrium reflex disturbed	0	-	-

The temperature factor significantly influences the rate of development of the intoxication process and its results. A comparison of the data in Table 1 and 2 shows that at 24°C, the death of the bulk of organisms ensues within 72 hours. At an initial temperature of 13.5°C with an increase to 17.5°C , the first signs of intoxication appeared considerably later. Only a repeated exposure to wastes (four exposures, 15 hours cumulatively) at intervals with detoxification periods of 10-15 days (total 36 cumulative days) lead to irreversible consequences for fish.

A short (6 minutes) exposure of roach (mean weight 16.7 g) to wastes caused a persistent loss of the equilibrium reflex in fish. In diluted wastes, this symptom appeared only in selected species.

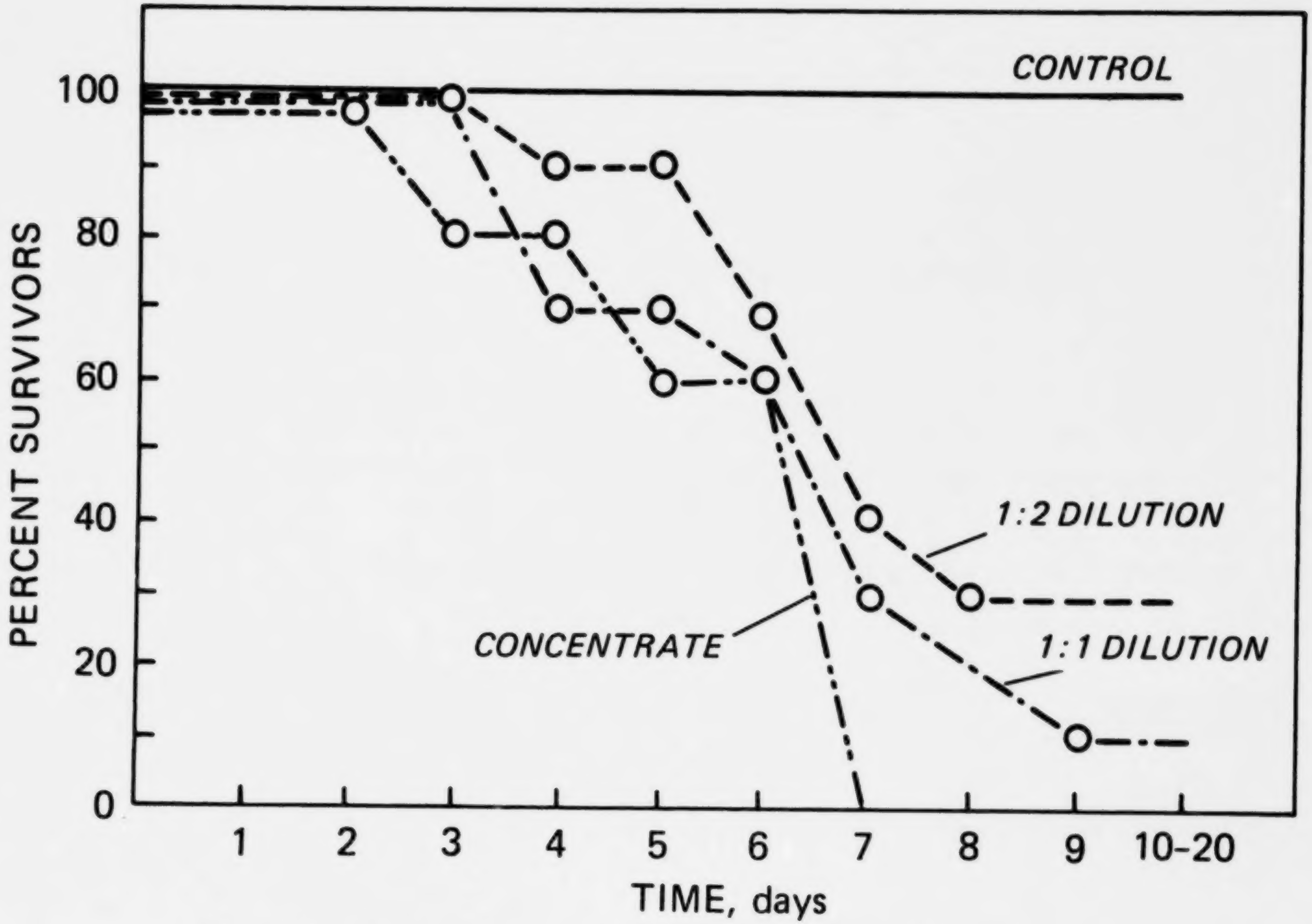

Figure 1. The dynamics of the survival rates of salmon larvae.
Age - 7 days
Mean weight - 98 mg.
Temperature - 11°C
The time of exposure - 6 min.

TABLE 2. REVERSIBILITY OF INTOXICATION IN JUVENILE SALMON DURING FOUR EXPOSURES TO EFFLUENTS DILUTED IN A RATIO OF 1:1
(Mean weight 147 mg, Age - 1 Month, Temperature 13.5 - 17.5°C)

First contact			Second contact			Third contact			Fourth contact		
Exposure time in solution (min)	Condition of fish after exposure	Survival of fish in clean water, % / 1-15 days	Exposure time in solution (min)	Condition of fish after exposure	Survival of fish in clean water, % / 16-26 days	Exposure time in solution (min)	Condition of fish after exposure	Survival of fish in clean water, % / 26-36 days	Exposure time in solution (min)	Condition of fish after exposure	Survival of fish in clean water, % / 37th day
194	Activity reduced, darkened skin	100-90	594	Activity reduced	90-60	75	Activity reduced	60-30	34	On the bottom, immobile, feeble respiration	Death within 13 min

By the end of the first day of detoxication in pure water, the state of the majority of fish did not differ from that of the controls. They actively swam, obviously reacted to external stimuli, and consumed food. The fish exposed to the point of equilibrium loss during intoxication, restored horizontal positioning in pure water only at intervals, ultimately sinking to the bottom and dying on the second day. The survivors did not differ from controls after 25 days of detoxification. They were again subjected to the action of the toxicants. During a repeated 5-minute exposure with concentrated sewage, the inverted position was observed. In diluted sewage, unstable reactions were noted, but an equilibrium state was recorded. In pure water, the roach exposed to the concentrates died on the third day, 20 percent of fish exposed to weak dilutions survived (Figure 2).

The dynamics of perch survival rate in pure water after 7 minutes exposure is illustrated in Table 3. A situation similar to that described above was observed when fish were exposed to concentrated and weakly diluted industrial wastes (boiling shop, evaporating and hydrolysis shops), and to the waters of a natural waste water receiver, the isolated bay of a reservoir.

Experiments determining reversibility of intoxication in fish after a brief exposure to effluents from a heat-and-power station were also revealing, since they are considered to be relatively pure by industry. After fish were exposed to effluents from a heat-and-power station diluted in ratios of 1:5, 1:10, and 1:25 for 6, 10, and 24 minutes, respectively, only a minor suppression of activity was observed. At the dilution 1:5 there was a thin coating of coal observed on the fins. Mortality during the 10 day period of detoxification was only 20 percent. However, additional exposure of fish at the same dilutions of wastes for 7, 16, and 24 minutes led to the death of the fish after 20 minutes in the first case, after a day in the second case, and only at a dilution of 1:25 did 40 percent of the experimental fish survive (Table 4). These examples convincingly demonstrate the high toxicity of treated wastes of sulphate pulp manufacturing.

The results of the experiment given in Table 5 are good evidence for the dependence of the result on the duration of exposure.

The data show that only a four minute difference in exposure marked effects in the outcome of intoxication.

The dependence of the reversibility rate on concentration in roach larvae is shown in Table 6.

As was demonstrated earlier, the main factors determining the resistance and degree of restoration of activity, are the duration of exposure and the concentration of the toxicant. This is also demonstrated in Table 7, which shows that the purified wastes from treatment plants loose their toxic properties to a considerable degree, and although there are some symptoms of intoxication, life activity is restored in pure water. Table 8 gives an indication of the reaction of juvenile fish of various species to toxicants.

Thus, an extensive investigation into the pattern of intoxication from effluents and its possible reversibility demonstrated that even brief expo-

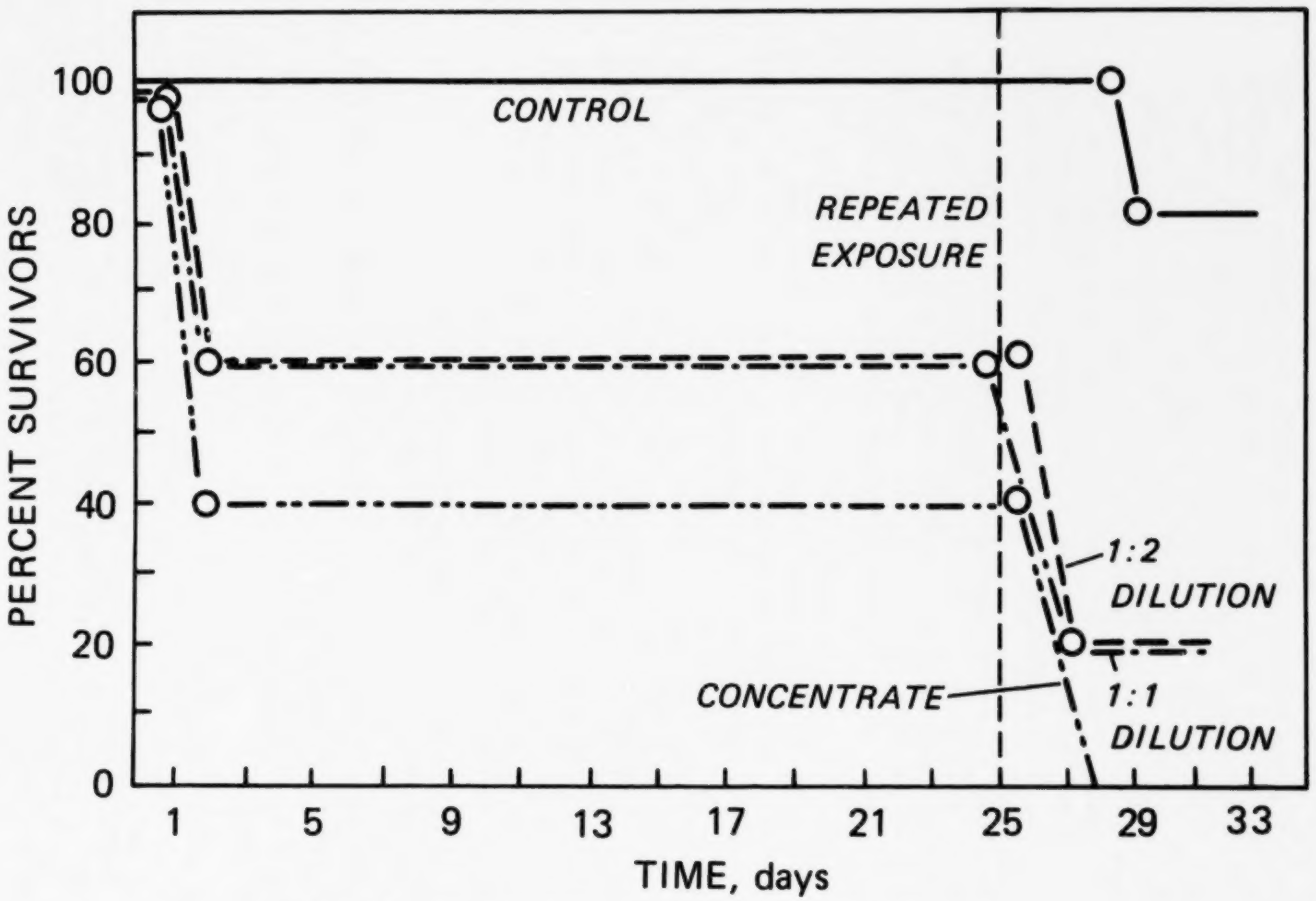

Figure 2. The dynamics of the survival rate of roach.
Mean weight - 16.7 g
Temperature - 17°C
The time of exposure - 1-6 min.
2-5 min.

TABLE 3. REVERSIBILITY OF INTOXICATION IN PERCH CAUSED BY EFFLUENTS
(Mean weight - 12.6 g, Temperature - 17°C, Exposure - 7 min.)

Dilutions	Condition of fish after exposure	Survival % in clean water by days													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14-26
Control	Swimming actively, reactive to stimuli	100	100	100	100	100	100	100	80	80	80	80	80	80	80
1:2	Very excited	100	100	60	60	60	40	40	40	40	40	40	40	40	20
1:1	One perch in lateral position, rest near the bottom	100	40	40	40	20	20	20	20	20	20	20	20	20	20
Undiluted waste	Float at the surface in lateral position	100	60	60	20	20	20	0	-	-	-	-	-	-	-

TABLE 4. REVERSIBILITY OF INTOXICATION IN JUVENILE SALMON CAUSED BY EFFLUENT FROM A HEAT-AND-POWER STATION
(Mean weight - 125 mg)

	First exposure			Second exposure		
Dilution	Exposure time (min)	Condition of fish after exposure	Survival in clean water, % 1-10 days	Exposure time (min)	Condition of fish after exposure	Survival in clean water, % 1-10 days
Control			100			100
1:25	24	Insignificant decrease in activity	100-80	24	Poorly mobile, "stand" on the bottom in vertical position	80-40
1:10	10	Insignificant decrease in activity	100-80	16	Poorly active, thin coating on fins	Death within 24 hrs
1:5	6	Increased activity, thin coating of carbon on fins	100-80	7	Equilibrium reflexes disturbed, coating on fins	Death within 20 min

TABLE 5. REVERSIBILITY OF INTOXICATION IN JUVENILE SALMON CAUSED BY EFFLUENT WATER (Dilution 1:5) FROM A HEAT-AND-POWER STATION

First exposure			Second exposure		
Exposure time (min)	Condition of fish after exposure	Survival in clean water 1-10 days	Exposure time (min)	Condition of fish after exposure	Survival in clean water 1-10 days
2	Fins slightly covered by coating of carbon	100-70	8	Carbon coating on fins, frequently "stand" on the bottom	60-30
6	"Stand" on the bottom, carbon coating on fins, convulsion of the body	40-30	8	Lie on the bottom, carbon coating on fins	Death within 30 minutes

TABLE 6. REVERSIbILITY OF INTOXICATION IN ROACH LARVAE CAUSED BY WASTE WATER FROM BOILING SHOP
(Mean weight - 24 mg)

	First exposure			Second exposure			Third exposure		
Dilution	Exposure time (min)	Condition of fish after exposure	Survival of fish in clean water, % 1-10 days	Exposure time (min)	Condition of fish after exposure	Survival of fish in clean water, % 11-20 days	Exposure time (min)	Condition of fish after exposure	Survival of fish in clean water, % 21-30 days
Control	-	-	100	-	-	100	-	-	100
1:10	90	Poorly active	100	150	Reduced activity, disturbance of schooling behavior	100	137	Activity decreased	93-87
1:5	60	Reduced activity, disturbance of schooling behavior	100	109	Poorly active, disturbance of schooling behavior, change of coloration	80-40	73	Poorly active, disturbance of schooling behavior	33
1:3	22	Disturbance of schooling behavior, loss of equilibrium change of coloration	63	90	Poorly active, disturbance of schooling behavior, change of coloration	73-47	8-19	Sluggish, keep at the surface, coordination disturbed	33-0

TABLE 7. REVERSIBILITY OF INTOXICATION IN SALMON LARVAE CAUSED BY WATER FROM AERATOR-TANK
(Mean weight - 103 mg, Temperature 7-21°C)

Dilution	First exposure			Second exposure			Third exposure		
	Exposure time (min)	Condition of fish after exposure	Survival of fish in clean water, % 1-10 days	Exposure time (min)	Condition of fish after exposure	Survival of fish in clean water, % 11-20 days	Exposure time (min)	Condition of fish after exposure	Survival of fish in clean water, % 21-30 days
Control	-	-	100	-	-	100	-	-	100
1:5	24	No visible symptoms of intoxication	100	24	No visible symptoms of intoxication	100	24	Insignificant increase in activity	100
Undilued waste	3.5	Increased activity, change of coloration (darkening)	100	24	Increased excitability, darkening	100	3.5	Decreased activity, darkening	100

TABLE 8. REVERSIBILITY OF INTOXICATION IN JUVENILE FISH OF VARIOUS SPECIES CAUSED BY UNDILUTED WASTE WATER

Species	Exposure duration (min)	Condition of fish after exposure	Survival % in clean water by days													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14-26
Perch	16	Loss of equilibrium, recovered in 2 min	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Roach	19	Activity reduced, lying laterally	93	93	80	80	80	80	Death within 2 hours							
Salmon	6	On the bottom, respiration markedly reduced	100	100	100	80	80	0								
Pike	16	Lying below surface, respiration feeble	Death within 17 minutes													
Cisco	37	On the bottom	Death within 11 minutes													

sures to sulphate-cellulose discharges, with subsequent migration to pure water does not guarantee fish safety. These factors are especially dangerous when combined with high temperature regimes.

While the general symptoms of intoxication can be identified, there are specific variations, depending upon the composition of the complex effluents, its concentration, the duration of exposure, and the species of the test organism.

ACKNOWLEDGMENTS

My thanks are due to the research workers of the Petrozavodsk State University named after O.W. Kuusinen; A.N. Ryzhkov, G.A. Tikka, and L.G. Tyutyunnik for their help in performing the experiments.

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SECTION 15

ASPECTS OF THE INTERACTION BETWEEN BENTHOS AND SEDIMENTS IN THE NORTH AMERICAN GREAT LAKES AND EFFECTS OF TOXICANT EXPOSURES

John A. Robbins[1]

The sediments of the North American Great Lakes are mostly overlain by well-oxygenated waters and support a diverse and abundant population of benthic (bottom-dwelling) organisms. Principal species include the freshwater shrimp, *Mysis relicta*; the amphipod, *Pontoporeia hoyi*; many species of Oligochaete worms such as *Tubifex tubifex* and *Limnodrilus hoffmeisteri*; the midge larvae *Chironomus anthracinus* and a variety of freshwater clams such as *Sphaerium* and *Pisidium* spp. Many of these organisms occur in great abundance throughout the Great Lakes. The deposit feeding Oligochaete worms occur in polluted harbors in numbers exceeding 1,000,000 m^{-2} (P. McCall, pers. comm.), and even in the profundal sediments of Lake Erie in densities approaching 50,000 m^{-2}. Characteristically, *Pontoporeia hoyi* occurs in densities on the order of 1,000 m^{-2} throughout much of the Great Lakes. In Lake Erie, as well as in the inshore areas of the other Great Lakes, Chironomid larvae densities are roughly 500 m^{-2} (P. McCall and D. White, pers. comm.). These organism densities represent an enormous biomass dwelling in or interacting with the sediments.

Not only are certain benthos an important link in the food chain, but many of them significantly affect the stratigraphy of sediments (Robbins *et al*. 1977) and the exchange of nutrients between sediments and water through such activities as burrowing, feeding, respiration, and excretion. As the fine-grained sediments are both the ultimate sink and a partial source (cf Remmert *et al*. 1977) of nutrients in the Great Lakes, the life activities of the benthos are likely to be an important factor in the nutrient cycle. If, in turn, the behavior, physiology, or mortality of benthos are affected by aquatic pollutants, there can be potentially novel and important effects on major nutrient cycles. While there has been considerable work done on the role of benthos in sediment mixing and exchange of substances across the mud-water interface in other lakes (see Petr, 1976 for a review), very little has been done in the Great Lakes. The aim of this paper is to illustrate the effects of selected benthos on particle and solute transport and to indicate some preliminary results of exposing benthos-sediment microcosms to toxic substances.

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STRATIGRAPHIC EFFECTS OF NATURAL POPULATIONS

In early attempts to interpret radioactivity profiles in sediments of the Great Lakes (Robbins and Edgington 1975) it became clear that significant mixing of material occurred over the upper 10 cm of sediment. From later work (Robbins *et al*. 1977) it was evident that the sediment mixing was due to the presence of benthic organisms. At two locations in Lake Huron, twelve cores of fine-grained sediment were taken for comparison of the vertical distributions of the naturally occurring radionuclide, lead-210, and fallout cesium-137 with the distributions of benthic macroinvertebrates. In the absence of mixing, the activity of lead-210 should decrease exponentially with sediment depth reflecting radioactive decay ($T_{1/2}$ = 22.26 yr) on burial. In actuality, the lead-210 activity was constant down to 6 cm in cores at one location and 95% of the total invertebrates occurred within the zone of constant activity. At the other location, the zone of constant activity was only 3 cm deep but more than 90% of the benthos were confined to it. In each case comparison of published tubificid reworking rates with sediment accumulation rates showed that the activities of benthos were able to account for the mixing of sediments. An example of the effect of sediment mixing on cesium-137 profiles is given in Figure 1 for a core from Lake Erie where the sedimentation rate is exceptionally high. The observed alteration in the radioactivity profile over that expected in the absence of steady-state mixing is consistent with the measured vertical distribution of benthos which at this location consists primarily of mature and immature Oligochaete worms. Studies of the distribution of natural and fallout radionuclides in cores from Lake Erie (Edgington and Robbins 1979), Lake Huron (Johansen and Robbins 1977) and Lake Michigan (Edgington and Robbins 1975) show that the mixing of surface sediments occurs widely in the Great Lakes.

It may thus be expected that altered patterns of sediment mixing resulting from exposure of benthos to aquatic pollutants could result in altered and possibly uninterpretable radioactivity and heavy metal profiles. From our studies (Robbins 1977) it is apparent that the time resolution with which lake-wide pollution changes can be reconstructed from sedimentary records is limited by benthic reworking (bioturbation). Increased benthos mortality would be likely to improve the long-term resolution because of the associated reduction in sediment mixing.

LABORATORY STUDIES USING RADIOTRACERS

To investigate the role of benthos in the transport of sediment particles in a controlled ans systematic way, experiments were set up in the laboratory using a particle-bound radiotracer, cesium-137. Illite clay particles with adsorbed cesium-137 were added as a submillimeter layer to the surface of fine-grained sediments contained in plastic cells of a rectangular cross section stored in a temperature-regulated aquarium. A well-colli-

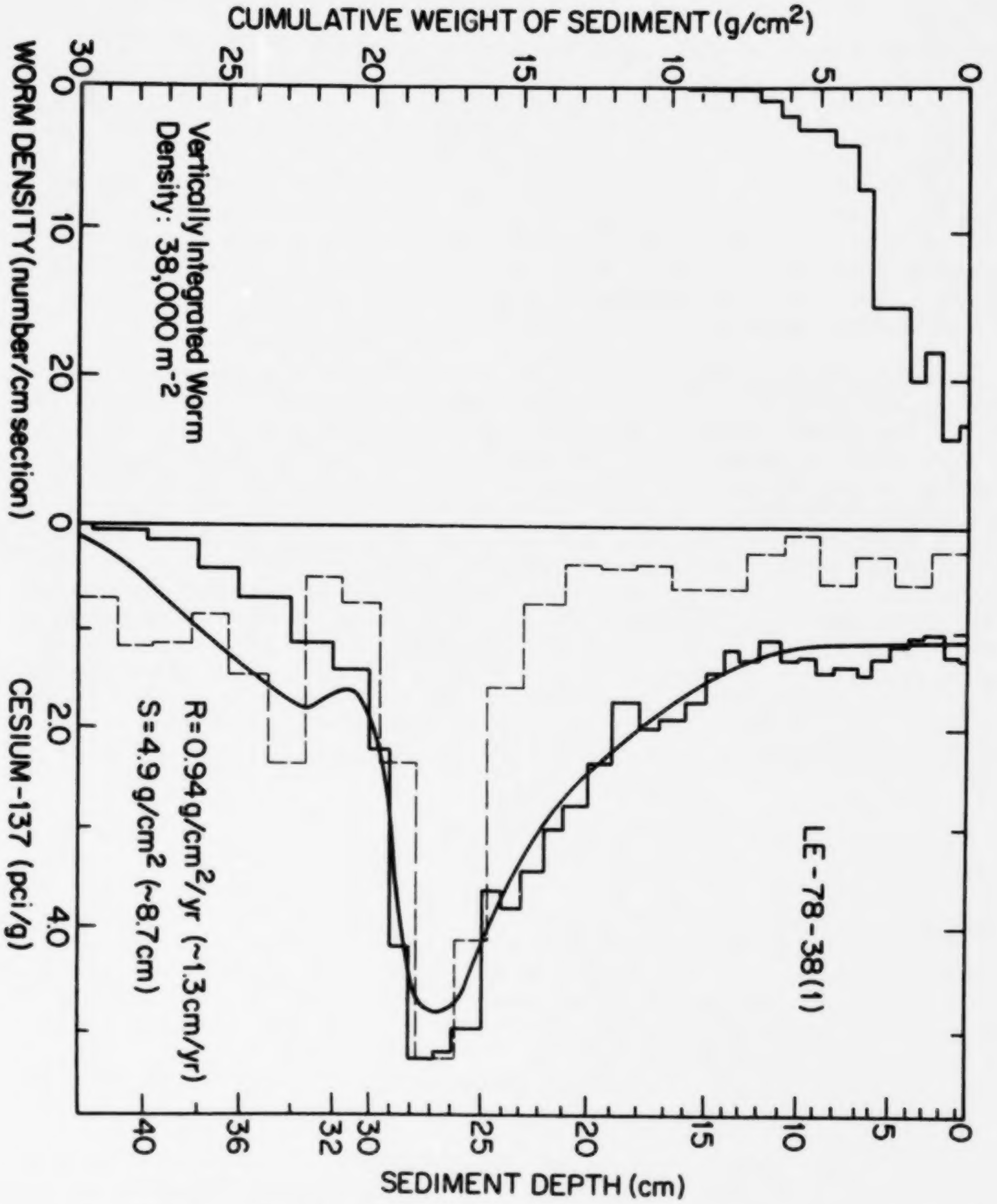

Figure 1. Distribution of benthos and cesium-137 in a core from Lake Erie. In the absence of sediment mixing the expected distribution of cesium-317 should reflect the history of atmospheric fallout (dashed line). The measured distribution is shown as the solid line histogram. The theoretical distribution shown as the continuous curve is based on the assumption of steady state mixing to a depth of 8.7 cm. The measured distribution of Oligochaete worms is consistent with this mixing depth. The results illustate the stratigraphic effects of benthos which are observed widely in the Great Lakes.

mated sodium iodide crystal gamma detector scanned the length of each of several cells at daily or weekly intervals over a period of several months in order to determine how several benthic species transported labeled particles away from the sediment surface. The experimental set up with an exposed aquarium containing several cells, a detector and a counting system are shown in Figure 2. Details of the construction and operation of the system are found in Robbins et al. (1979). The actual and measured distribution of activity from a submillimeter line source is shown in Figure 3. The nearly Gaussian profile of measured activity mainly reflects collimator geometry. The limited broadening of the line source in the control cell (with no benthos present) is due to molecular diffusion.

When Oligochaete worms are added to surface-labeled sediments, the radioactivity profile evolves over a six-month period as illustrated in Figure 4. The shaded areas represent the profile corrected for the effects of finite detector resolution. The initial effect of the worms on the distribution is one of burial. This is, of course, consistent with the well-known behavior of these organisms. They penetrate sediments to about 10 cm depth to feed while at the same time holding their tails above the sediment surface to defacate. This behavior has led Rhoads (1974) to describe such organisms as "conveyor-belt" species. In time, the marked layer is buried to the point where it encounters the zone of feeding and begins to reappear at the sediment surface. During the initial burial period, the reworking rate is essentially constant as can be seen in Figure 5 which shows the location of the peak activity versus time. The burial rate is about 0.052 ± 0.007 cm/day at 20 degree C. Error bars primarily reflect uncertainty in locating the sediment-water interface due to irregular pile up of fecal mounds.

The interaction of the amphipod, Pontoporeia hoyi, with sediments strongly contrasts with that of Oligochaete worms. As can be seen in Figure 6, the activity spreads downward from the surface under the action of Pontoporeia without significant advection. This species burrows randomly through the upper several centimeters of sediment and thus serves to move sediment particles in a manner akin to eddy diffusion. Shown in Figure 7 are the corrected peak width versus time plus a theoretical relationship based on the assumption that particle motion is truly eddy diffusional in character. Details of the calculation are given in Robbins et al. 1979. The diffusion coefficient implied by the data is 4.4 cm^2/yr for an amphipod density of 16,000 cm^{-2}.

While the two benthic species investigated have very different modes of interaction with sediments, their effect on vertical particle movement can in each case be quantitatively described and measured with a precision and rapidity which suggests the radiotracer method as a useful behavioral bioassay technique. Very precise reworking rates, expressed either in terms of a sediment burial rate or eddy diffusion coefficient, can be determined under realistic conditions in a matter of a few days. This radiotracer method of observing a particular organism's behavior offers the special advantage of being noninteractive to a very high degree. The gamma radiation passes readily through the cell walls and, once radionuclides have been added to the system, no further interaction with the microcosm is required

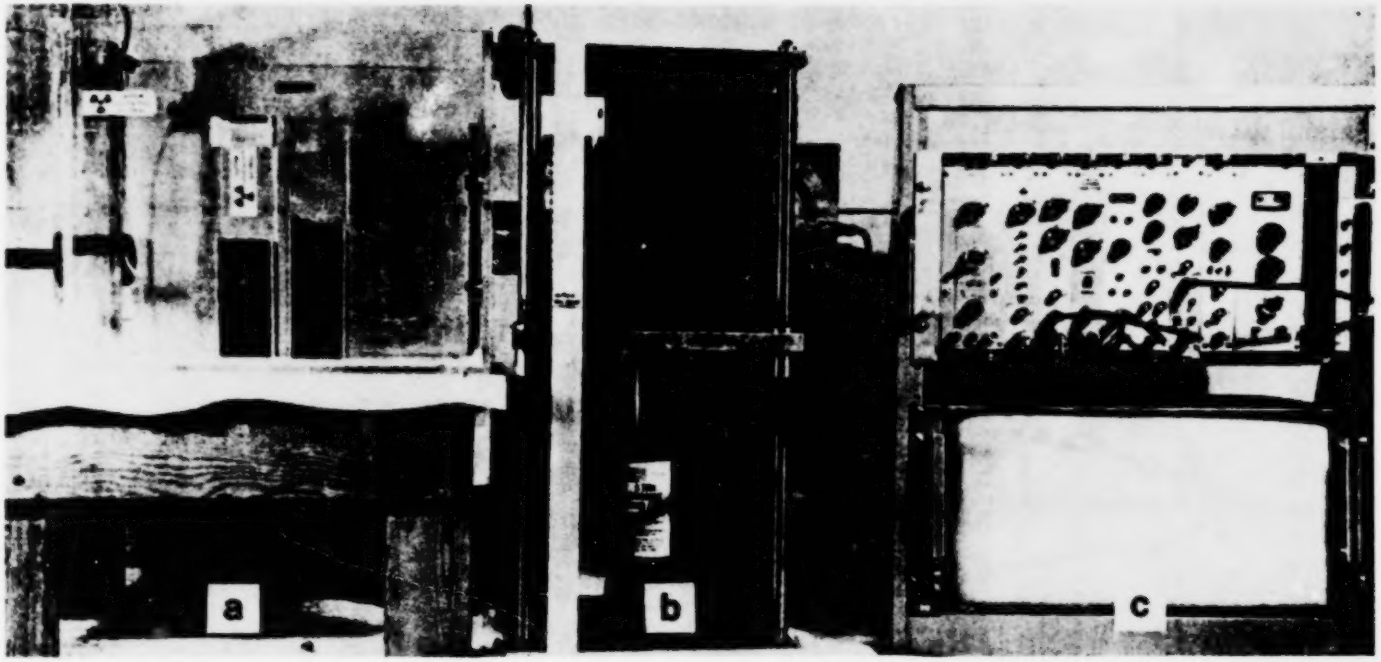

Figure 2. The radiotracer scanning system. (a) Aquarium with experimental cells; (b) shielded detector and frame; (c) counting system.

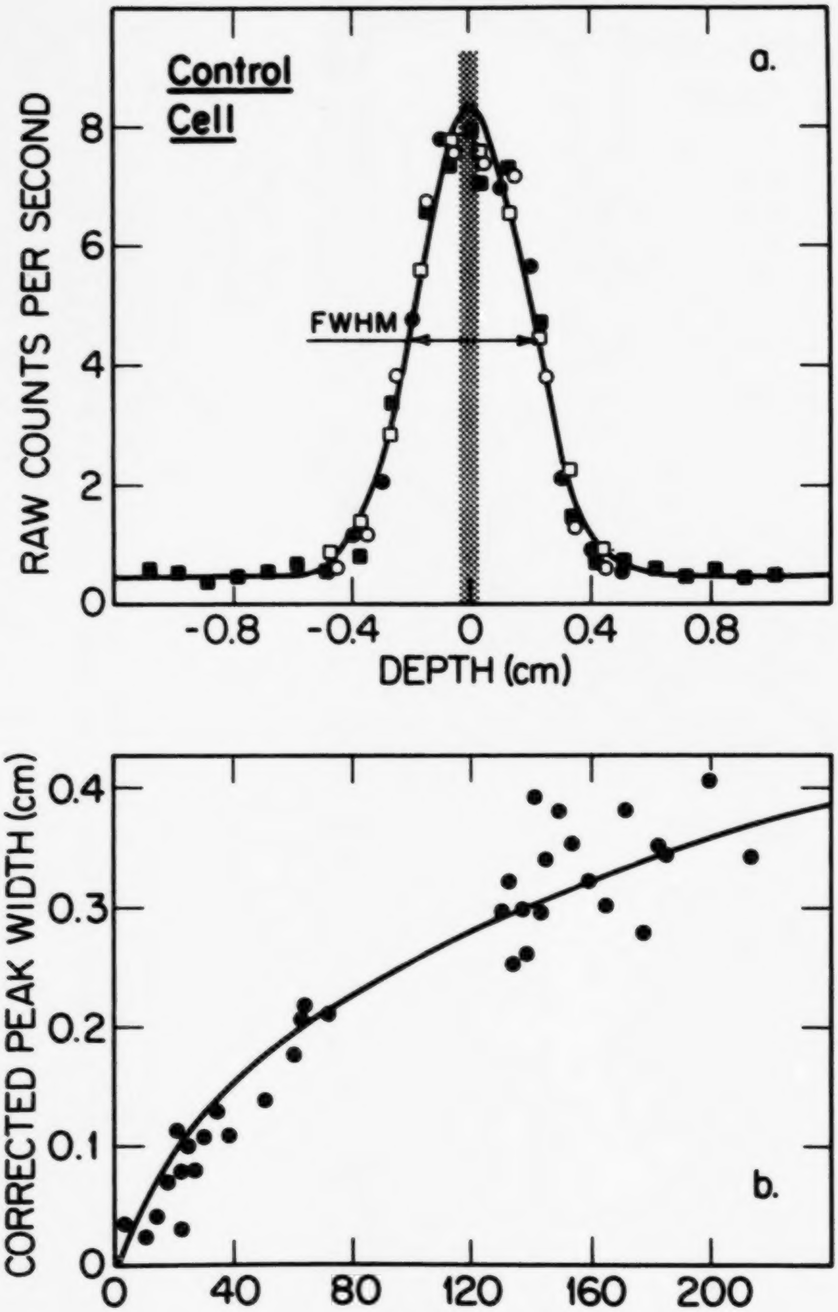

Figure 3. (a) The actual and measured distribution of activity from a submillimeter line source. The finite width of the profile is a result of detector optics. (b) The limited broadening of the line source in time is due to molecular diffusion and is small in comparison to that introduced by benthos.

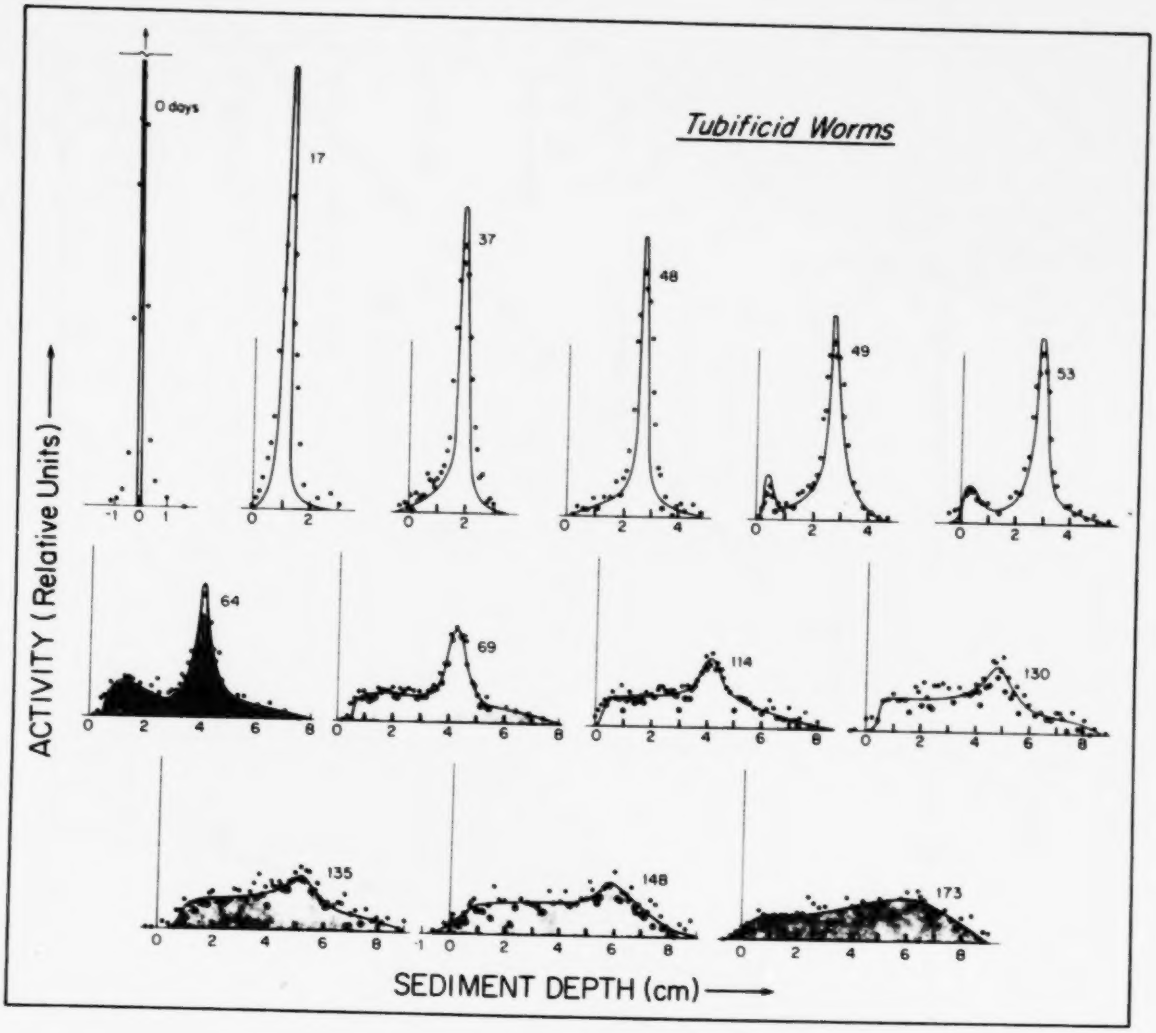

Figure 4. Effect of tubificid worms on the distribution of cesium-137. Shaded areas are the activity profiles corrected for system optics. Vertical lines indicate the location of the sediment-water interfaces.

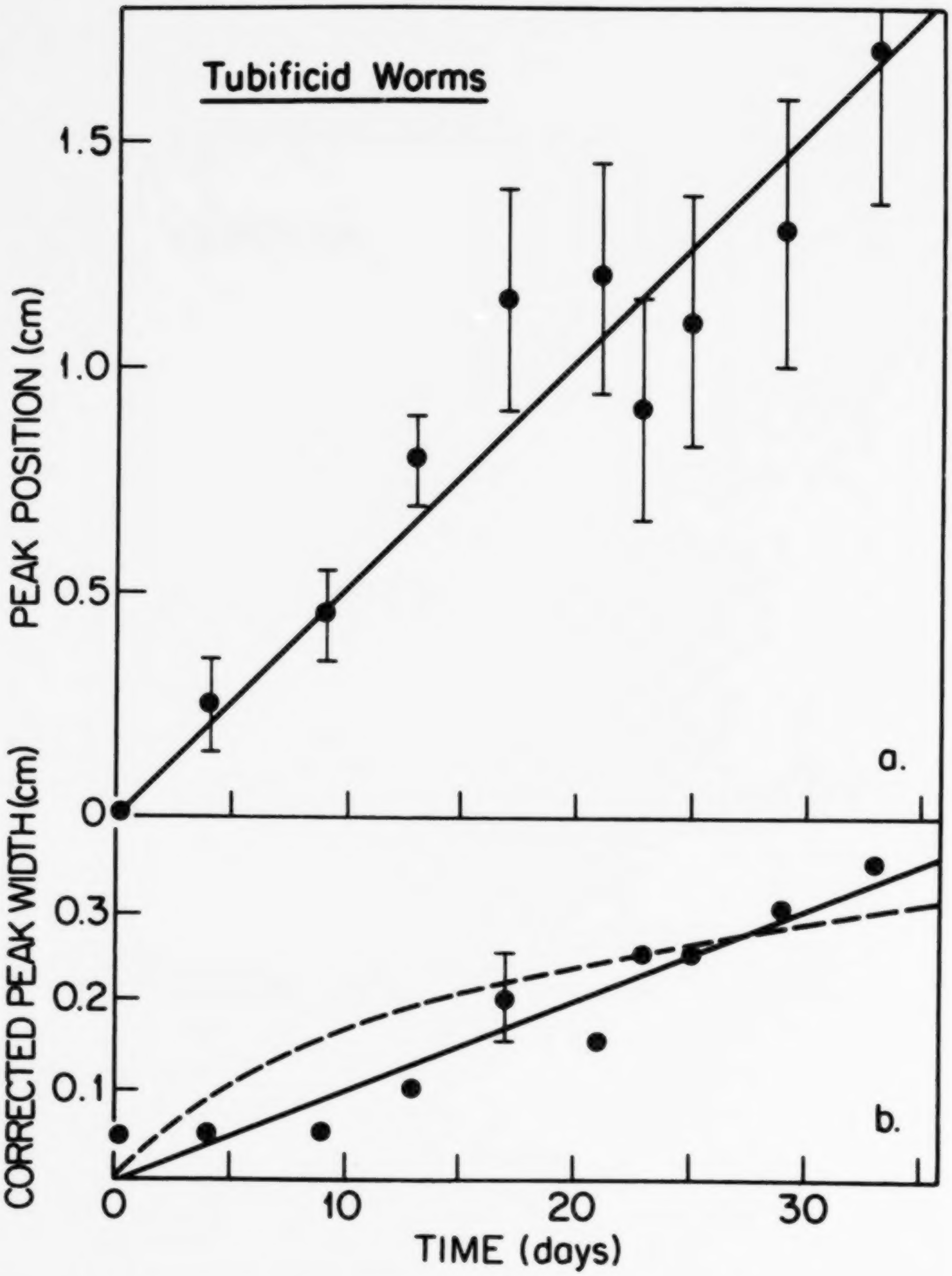

Figure 5. (a) Location of the peak activity versus time. The rate of burial is essentially constant over the first month of observation. (b) Peak width corrected for system optics versus time. Dashed and solid lines are theoretical treatments of the peak broadening (see Robbins *et al*. 1979).

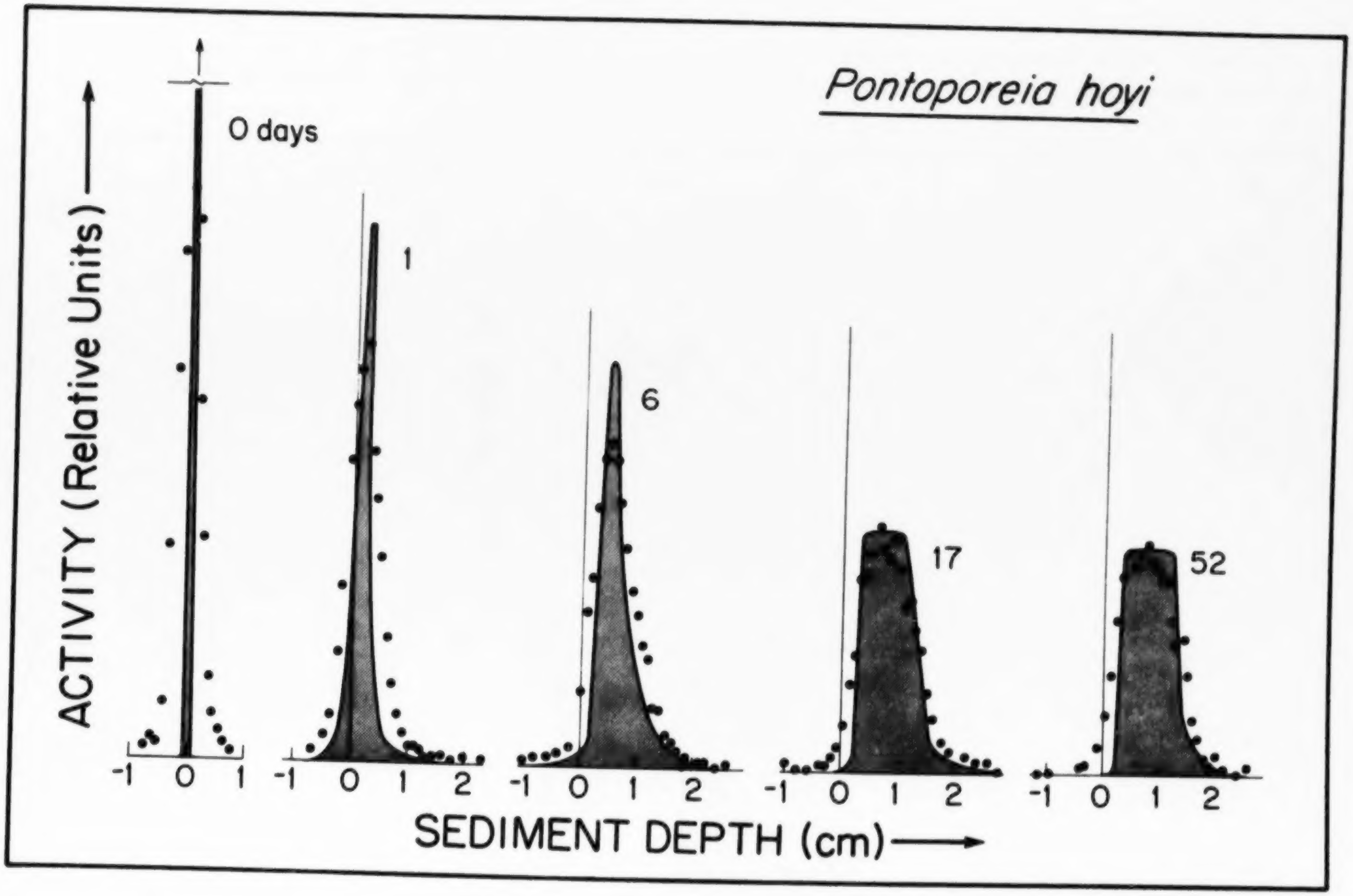

Figure 6. Effect of amphipods (Pontoporeia hoyi) on the distribution of cesium-137.

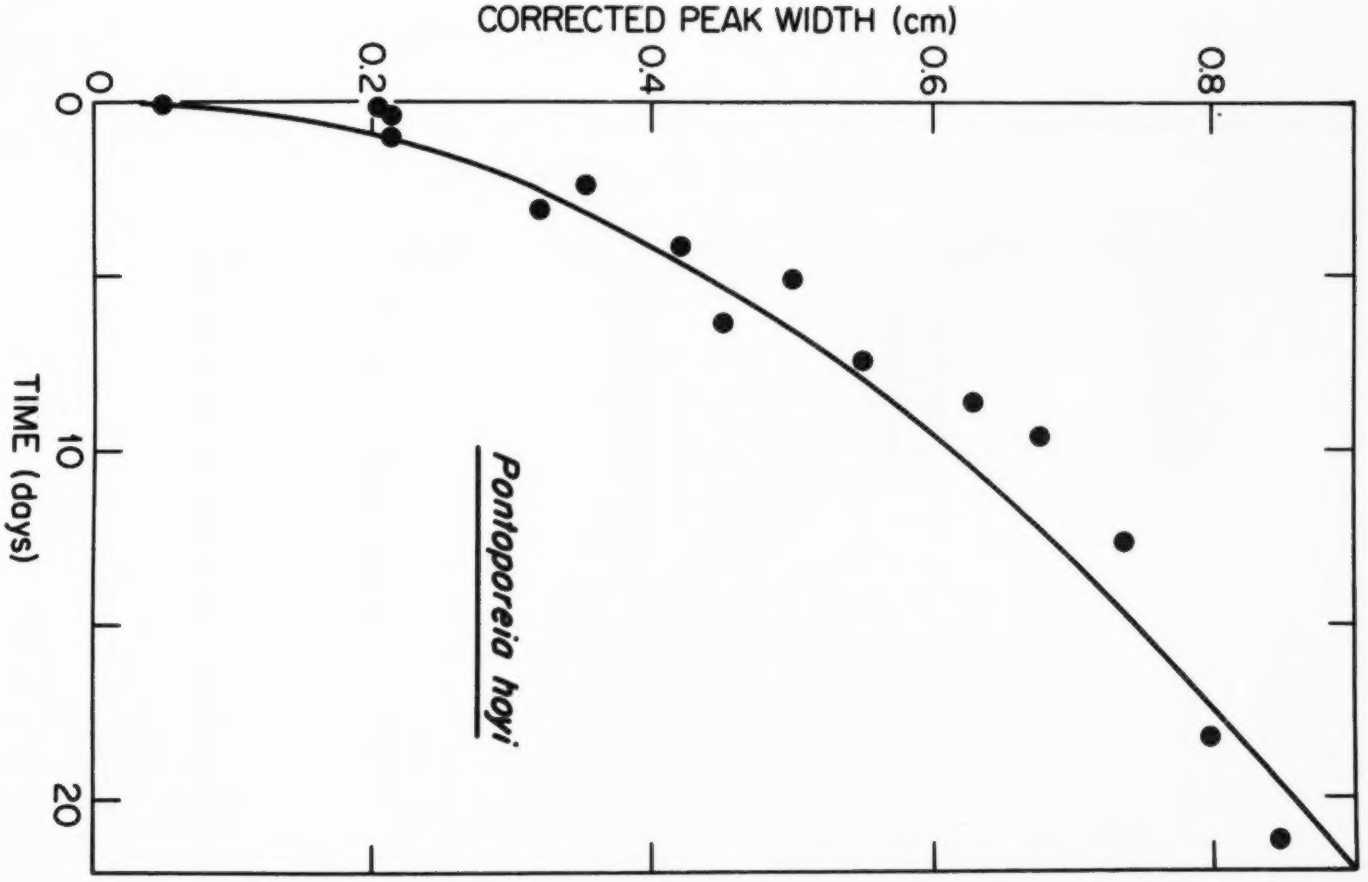

Figure 7. Time-dependence of the optics-corrected activity profile width (full width at half maximum). The solid line is the expected dependence if mixing of sediment solids occurs by eddy diffusion down to about 1.5 cm depth.

to make quantitative observations of processes occurring within it. The notion of using radiotracers to measure aspects of behavior in a noninteractive way can be extended to include species other than benthos, other behaviors, and other aquatic microcosms.

So far, we have not applied the method for carefully controlled assay of toxic substances but only for several trivial cases where it was important to demonstrate that the addition of major ions to water overlying cells would not affect reworking rates. Still, general features of the experiments are useful to consider. Either sulfate (SO_4) or chloride (Cl) ions were added as Na_2SO_4 or NaCl to a series of cells containing Oligochaete worms and a surface-labeled layer of sediment. Prior to addition of the ions, the reworking rate in each cell had been measured with the scanning system. Results are shown in Figure 8 for addition of NaCl. Below 5000 micrograms Cl/ml (ppm) no change in reworking rate was observed while the rate decreased abruptly following addition of NaCl at a concentration in overlying water of 10,000 ppm. In this case, the reduction was probably not a behavioral but rather a mortality effect. The results for sulfate are given in Figure 9 for two species of Oligochaete worms, Tubifex tubifex derived from Lake Michigan sediments and laboratory culture of Limnodrilus hoffmeisteri. The ratio of final to initial reworking rate is shown versus concentration. Again, significant decreases in reworking rates occur only for the very high concentrations used in the experiment. These levels of course far exceed any encountered in most lakes. There appear to be significant differences in the response of the two Oligochaete populations to SO_4 additions. More important experiments will involve additions of metals and toxic organics to these microcosms. Such work would represent a continuation of studies by others, notably Brkovic-Popovic and Popovic who have investigated the effect of heavy metals on survival (1977a) and on respiration rate (1977b) of tubificid worms. Problems will arise in the interpretation of the effects of nonconservative substances on the system, which were far less significant in the case of conservative ions like sulfate and chloride. Nonconservative materials may rapidly adsorb to sediment particles and little meaning may be attached to the concentration in overlying water. As sophistication develops in the use of such radiotracer behavioral assay methods, it will be desirable to take the community approach as there is considerably evidence for species interaction effects (Petr 1976). In related studies, it would be desirable to look at the relation between toxic substance exposure and the ability of benthos to avoid predation (Hall et al. 1979). A further effect which can be studied with relative ease with this method is the response of benthos to chronic oxygen depletion. Under conditions of oxygen depletion, feeding of tubificid worms is minimal. In sediments of Lake Easthwaite, worms spend most time at the mud-water interface (Stockner and Lund 1970) but resume feeding with the restoration of aerobic conditions.

The scanning method described above can also be used to investigate the effects of benthos on interstital transport. By using both a particle-bound radiotracer such as cesium-137 ($K_d \sim 5000$, Robbins et al. 1977) and a relatively conservative gamma emitting isotope, sodium-22 ($K_d \sim 2$, Lerman and Weiler 1970), reworking rates and molecular diffusion rates can be determined simultaneously. In a prototype experiment, we have investigated the

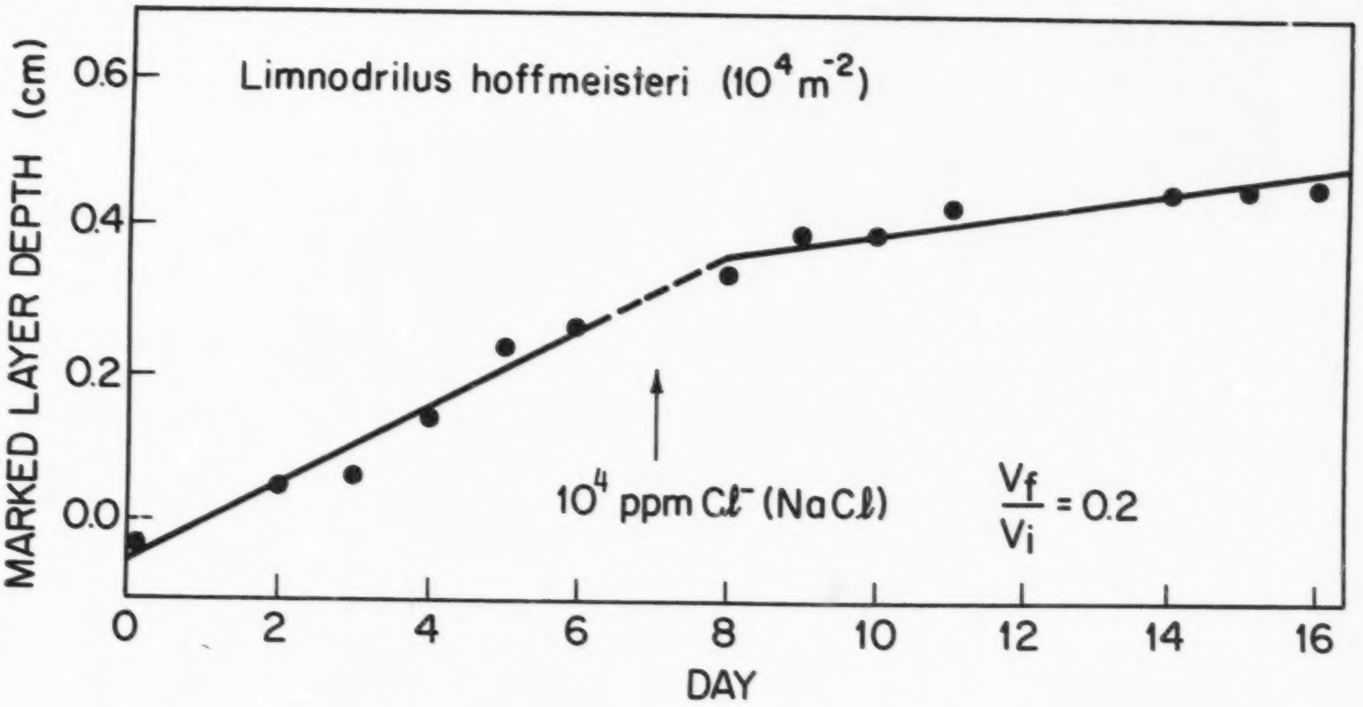

Figure 8. Effect of adding very high levels of NaCl (10,000 ppm Cl) on the rate of sediment reworking by the Oligochaete worm, Limnodrilus hoffmeisteri. A factor of five reduction results from this addition.

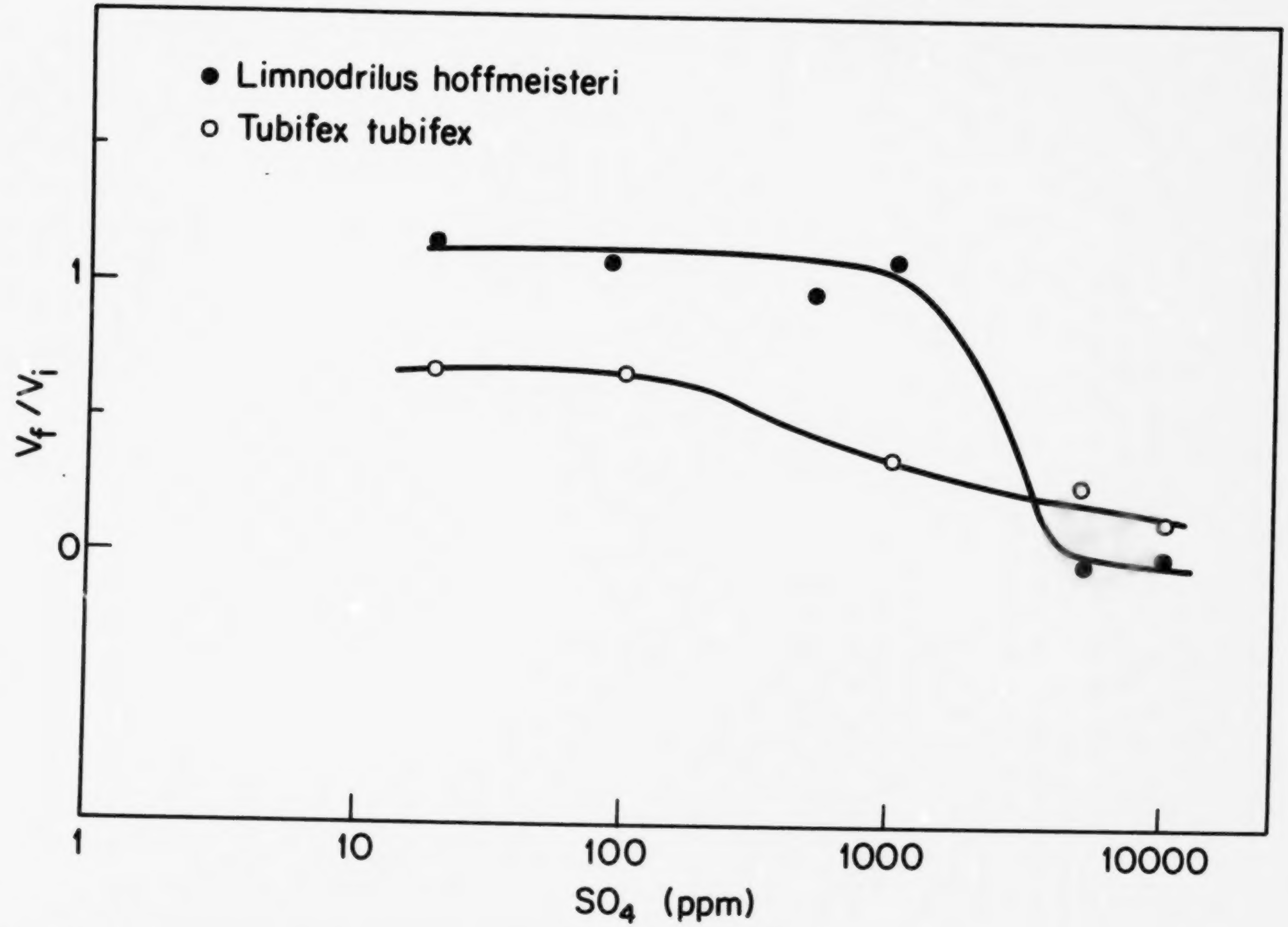

Figure 9. Response of the sediment reworking rate to additions of sulfate (Na_2SO_4) for two species of Oligochaete worms. Low values of the ratio of final to initial reworking rates for _Tubifex tubifex_ are probably not related to additions of sulfate but rather to mortality effects.

effect of Oligochaete worms on soulte transport. In each of two cells containing natural sediment and lake water, we added a submillimeter layer of cesium-labeled sediment and about 20 microCuries of sodium-22 as NaCl. To one cell we then added worms to achieve a density of about 70,000 m^{-2}. Following this treatment the two cells, control and worm, were scanned about once a day for over 10 days. The results of the experiment are illustrated in Figure 10. Profiles of cesium-137 and sodium-22 are shown after an elapsed time of about 200 hours. In the control cell, there is no significant displacement of the marked layer while in the worm cell, the layer has moved downward by an amount corresponding to a rate of about 0.055 cm/day. In the worm cell, the Na-22 has penetrated further into the sediments than in the control. Note that the measurements in the worm cell were made about 40 hours earlier than in the control. Thus the downward movement of the sodium-22 would be even more pronounced if the profiles could have been taken at the same time. The solid curve is the expected distribution of sodium-22 based on a solution to the diffusion equation with values of the diffusion coefficient chosen to give the best least squares fit to the data. In the control cell, the effective diffusion coefficient is 3.9×10^{-6} cm^2/sec while in the worm cell, the value is 13.1×10^{-6} cm^2/sec. Thus, the presence of tubificid worms at a density of about 70,000 m^{-2} enhances the diffusion coefficient by over a factor of 3. In a separate experiment where the sediments had been conditioned by allowing worms to create an equilibrium system of burrows, but where there was no active reworking at the time of adding radiotracers, the diffusion coefficient for Na-22 transport was still enhanced (x2) over its value in a control cell having no conditioned sediments. Therefore, it seems that the enhancement of pore water diffusion by tubificid worms results from their loosening of the sediments through the creation of a system of burrow channels rather than to their momentary life activities. Thus, the short-term effect of reducing or terminating the burrowing activity of worms through exposure to aquatic pollutants would seem to be small but the long-term result would appear to be the collapse of the burrow structure with an associated reduction in the ability of ions to migrate through pore fluids.

With proper experimental design, the radiotracer method could be used to examine the effect of aquatic pollutants on benthos-mediated transport of solutes. However, a more direct approach is to relate measured sediment-water fluxes to the density of activities of benthos.

NUTRIENT FLUXES FROM UNDISTURBED SEDIMENT CORES

We have taken this approach in collecting a series of cores from various locations in the Great Lakes (Remmert _et al_. 1977; Robbins _et al_. 1976). Undisturbed 7.6 cm diameter cores of fine-grained sediments from Lakes Michigan, Huron, and Erie were stored in _in situ_ temperatures (∿60° C) in their original plastic liners along with about 10 cm of overlying water. Increases in the concentration of reactive dissolved silica over periods of hours to days in stirred, oxygenated overlying water provided estimates of the rate of exchange of dissolved silicon across the sediment water interface. The increases in the concentration of silicon (ppm Si) versus time is shown for a core from Saginaw Bay, Lake Huron in Figure 11. The release

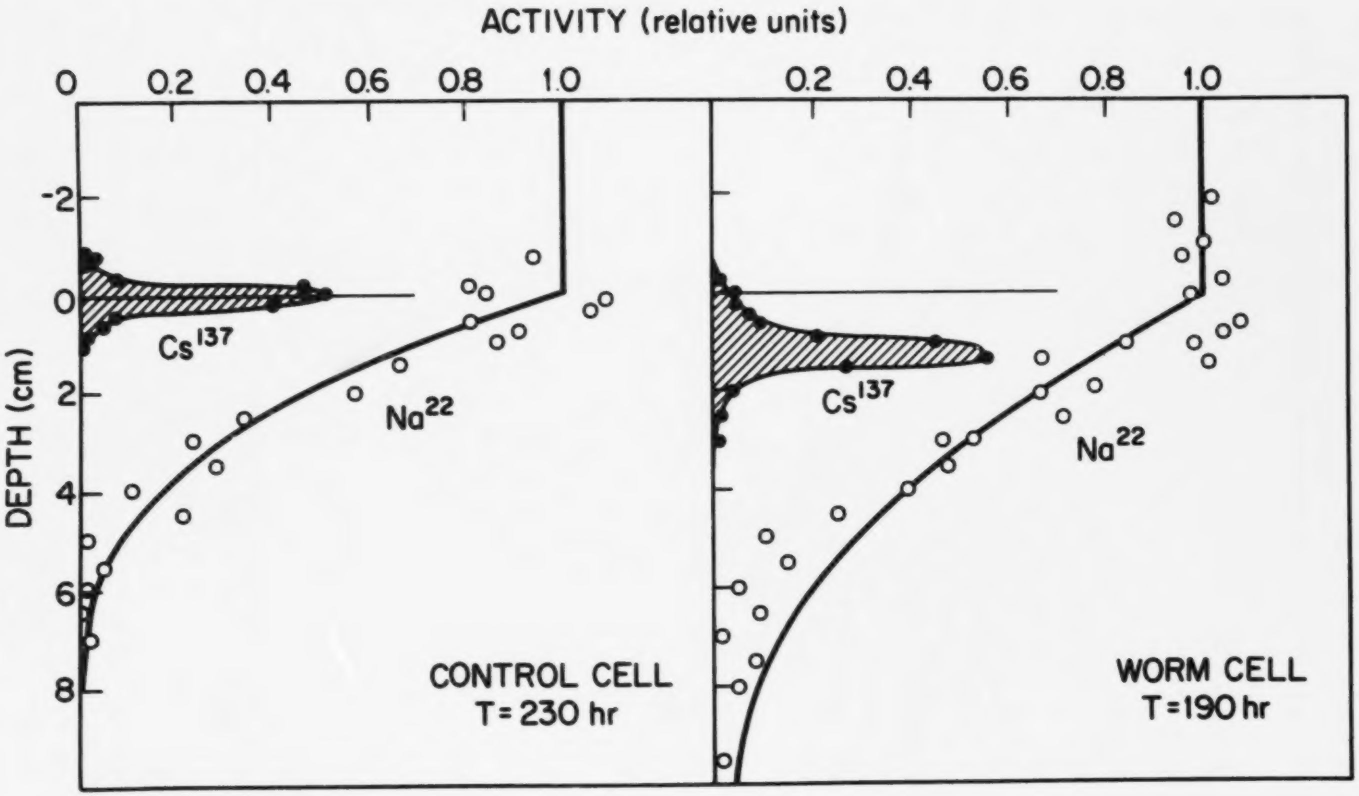

Figure 10. Activity of cesium-137 and sodium-22 in a control cell and in a cell with tubificid worms after an elapsed time of about 200 hours. In the control cell there is no displacement of the particle-labeled surface layer while in the worm cell, the labeled layer has been buried about 1 cm. In both cells, the sodium-22, initially added to overlying water, has migrated an appreciable distance into sediments, but in the worm cell, the migration is significantly more rapid. Solid lines represent solutions to the diffusion equation. Worms enhance the effective diffusion coefficient by about a factor of 3 at densities of about 70,000 m^{-2}.

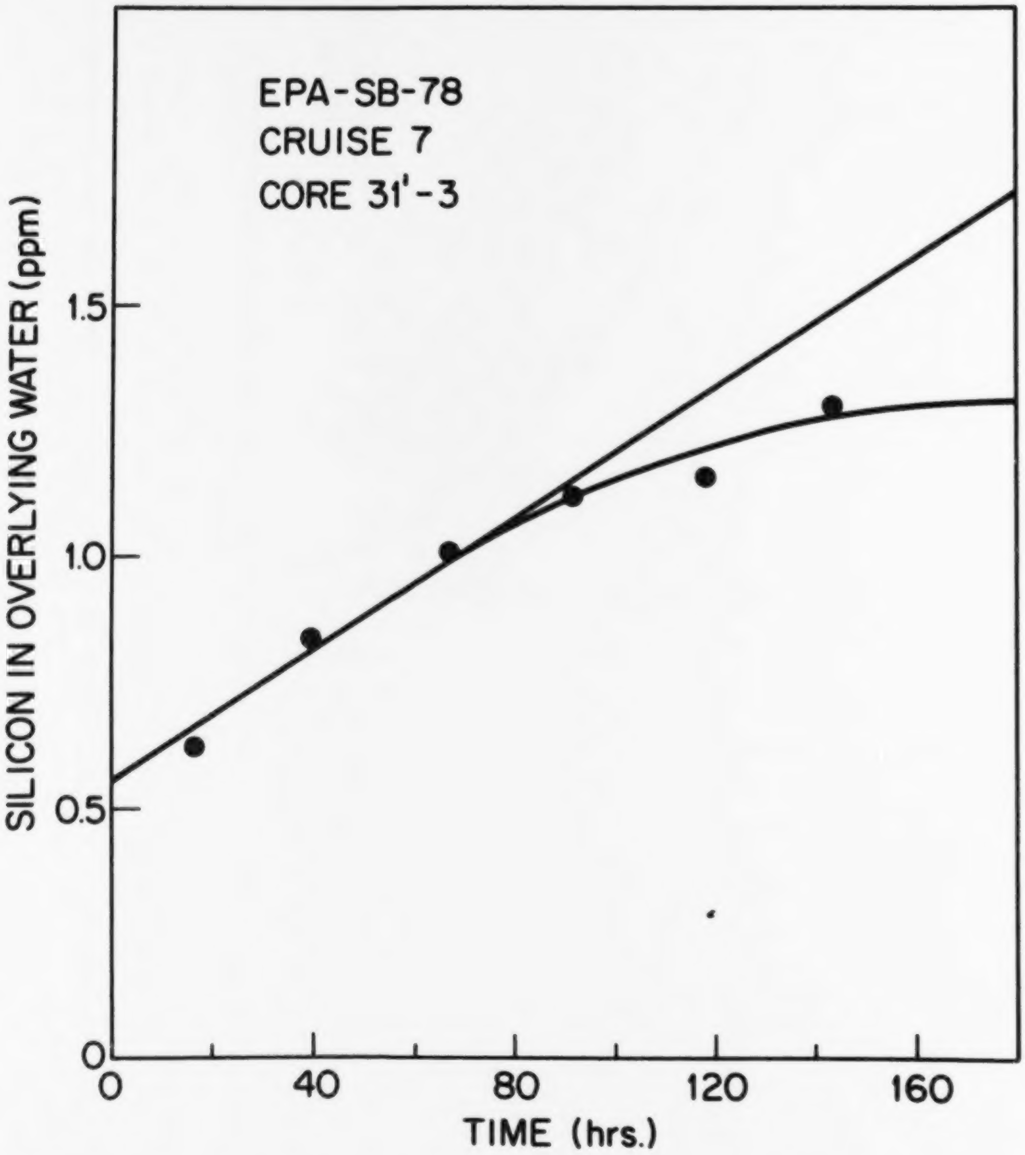

Figure 11. Concentration of soluble reactive silicon in water overlying sediments stored without disturbance in a core liner collected from Saginaw Bay, Lake Huron. Over about the first hundred hours, the release of Si into overlying water is essentially constant.

rates for each of the lakes was similar despite different seasons of coring each lake and averaged about 2000 (i.e., 2000 μg Si/cm^2/yr) micrograms Si/cm^2/yr. If this flux represents an annual average then the amount of Si regenerated from sediments each year in Lake Erie for example is enormous. The vertically integrated amount of dissolved silicon in the water column is a maximum of about 200 micrograms/cm^2, so the time required to replenish the Si removed from the water (through incorporation into diatoms) by regeneration from sediments is 0.1 year. Robbins and Edgington (1979) found that the flux of Si from sediments in Lake Erie is proportional to the concentration of amorphous silicon in surface sediments suggesting the flux is dominated by dissolution of particulate cilica recently deposited on the sediment surface. This result indicates a particular role for organisms like the larvae of Chironomids which are shallow water plankton detritus feeders and whose effect on the release of silicon from sediments was noted many year ago by Tessenow (1964).

By comparing silicon fluxes with benthos densities in a series of replicate cores taken on two cruises in Saginaw Bay, Lake Huron, last year (fall 1978), we have been able to confirm Tessenow's observations for our particular Great Lakes environment. Shown in Table 1 is the density of benthos in each of several replicate cores along with the silicon flux measured via timed sampling of overlying water as described above. It can be seen that the dominant species in terms of numbers are the immature tubificid worms. However, densities of these organisms correlate poorly with the silicon flux as can be seen from Table 2. Because of the limited number of observations most correlations are not significant. However, the correlation between the Si flux and the density of Chironomids is outstandingly high and significant for both observation periods (Figure 12). In this experiment, other nutrients were measured as well and correlations which are persistently high over both cruises are underlined. The observed decrease in the concentration of

TABLE 1. BENTHOS DENSITY AND SILICON FLUX: SAGINAW BAY, LAKE HURON

		Density (m-2)				
		Tubificids				
Cruise	Core	Mature	Immature	Naididae	Chironomids	Silicon Flux (μg/cm^2/yr)
7[1]	1	850	40,000	5900	0	1100
	3	850	65,000	7900	0	770
	1'	280	8,200	850	560	1800
	2'	1700	29,000	8200	850	2700
	3'	2300	18,000	560	560	1680
8[2]	1	1400	23,000	0	1130	3600
	2	280	5,600	0	0	1300
	8	280	5,600	0	280	1600
	2'	280	29,000	13000	560	2400

[1] October, 1978.
[2] November, 1978.

TABLE 2. CORRELATIONS BETWEEN NUTRIENT FLUXES AND ORGANISM DENSITIES

(CRUISE 7)

Organism Group	Phosphate (PO_4)	Ammonia (NH_3)	Nitrate (NO_3)	Sulfate (SO_4)	Silicon (Si)
Tub. Mature	0.93	-0.09	-0.17	-0.75	-0.07
Immature	0.07	-0.82	0.60	-0.54	-0.36
Naididae	-0.19	-0.49	0.13	-0.41	-0.29
Chironomids	0.11	0.74	0.04	0.04	0.97
Total	0.06	-0.69	0.55	-0.56	-0.26

(CRUISE 8)

Organism Group	Phosphate (PO_4)	Ammonia (NH_3)	Nitrate (NO_3)	Sulfate (SO_4)	Silicon (Si)
Tub. Mature	0.41	0.92	0.23	0.97	0.88
Immature	0.23	0.14	0.93	0.22	0.49
Naididae	-0.30	0.63	0.78	-0.50	0.13
Chironomids	0.09	0.63	0.25	0.76	0.99
Total	-0.9	-0.05	0.94	0.01	0.62

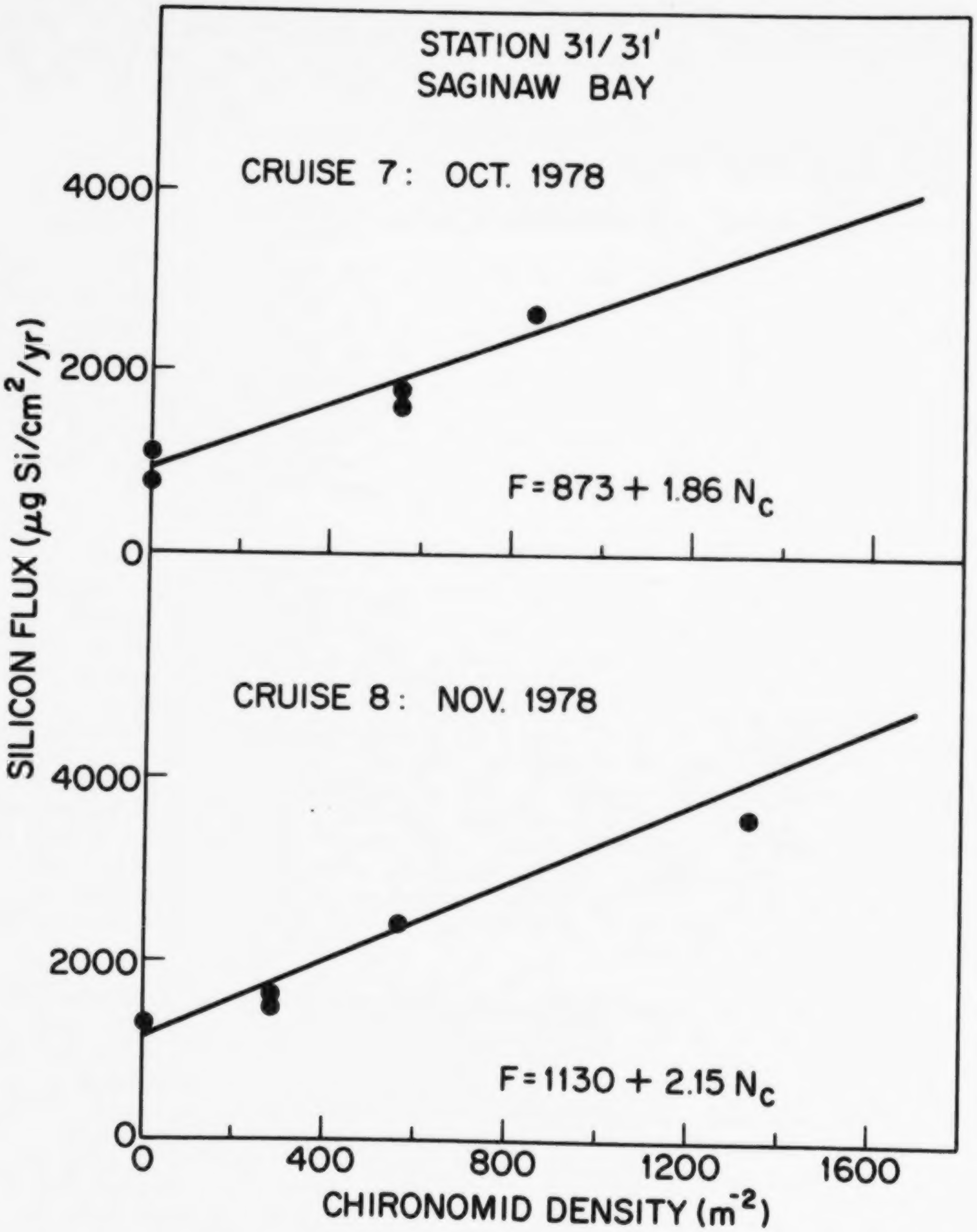

Figure 12. Relationship between the flux of Si from sediments and the density of Chironomid larvae in a series of replicate cores taken from Saginaw Bay, Lake Huron, on two separate cruises in 1978.

phosphate in overlying water is marginally associated with the presence of mature tubificid worms, the increase in ammonia is persistently associated with Chironomids, and the reduction in nitrate levels over time appears to be associated with the population of immature tubificids and/or the total macrobenthos population.

The results for silicon suggest the relationship:

$$\text{Flux} = 1000 + 2 \times \text{Chironomid larvae density},$$

where the flux is in micrograms Si/cm^2/yr and the density is in numbers m^{-2}. As the mean density of Chironomid larvae at this location is about 500 m^{-2}, roughly half the flux of silicon from the sediments is attributable to the presence of these organisms. This circumstantial evidence for the effect of Chironomids is strengthened by considering Tessenow's experiments with sediments from Lake Heiden, Germany (Tessenow 1964) in which he demonstrated a casual relationship. Addition of Chironomids (Pulmosus group) to his sediments resulted in enhanced silicon release. Converting Tessenow's results to the above form, we find that for his experiments:

$$\text{Flux} = 1000 + 4 \times \text{Chironomid larvae density}.$$

Graneli (1977) has also observed that *Chironomus Pulmosus* larvae increase the release of silica as well as phosphorus from sediments of several lakes in Sweden. It would therefore seem likely that at least in shallow waters of the Great Lakes where fine-grained sediments can be found, such as lower Saginaw Bay, and in most of Lake Erie, Chironomid larvae may play a major role in the regeneration of silicon from sediments. In Lake Erie, average Chironomid densities may be as high as 1000 m^{-2} (P. McCall, pers. comm.). That these organisms may enhance silicon fluxes does not necessarily mean that their removal or inhibition through exposure to aquatic pollutants will result in a long-term reduction in the capacity of the sediments to return silicon to overlying waters. It is always possible that the ecological niche represented by diatom detritus processing can be filled by another biotic or abiotic component. In other words, the role of Chironomid larvae may be mainly a kinetic one.

Several preliminary experiments have been undertaken to determine the effect of removing the influence of macrobenthos on release of silicon. A method must be chosen which results in minimal alteration of the structure or composition of sediments. In one experiment, a core incubated at *in situ* temperatures was exposed to 5 megaRads of cobalt-60 gamma radiation, enough exposure to completely sterilize the sediment core and overlying water. The results of this experiment are shown in Figure 13. Prior to irradiation, the silicon flux was 2000 micrograms Si/cm^2/yr. After irradiation, the flux dropped to 900 micrograms Si/cm^2/yr. It is interesting to note that the factor of two reduction in flux is consistent with the relation given above for the flux as a function of Chironomid larvae density. In this particular core, the density of benthos was not measured. A major reduction in the silicon flux also resulted from addition of Chlordane in amount sufficient to destroy the macrobenthos population (about 1 ml of Chlordane in a dispersant). Results of this and other treatments are given in Table 3. No

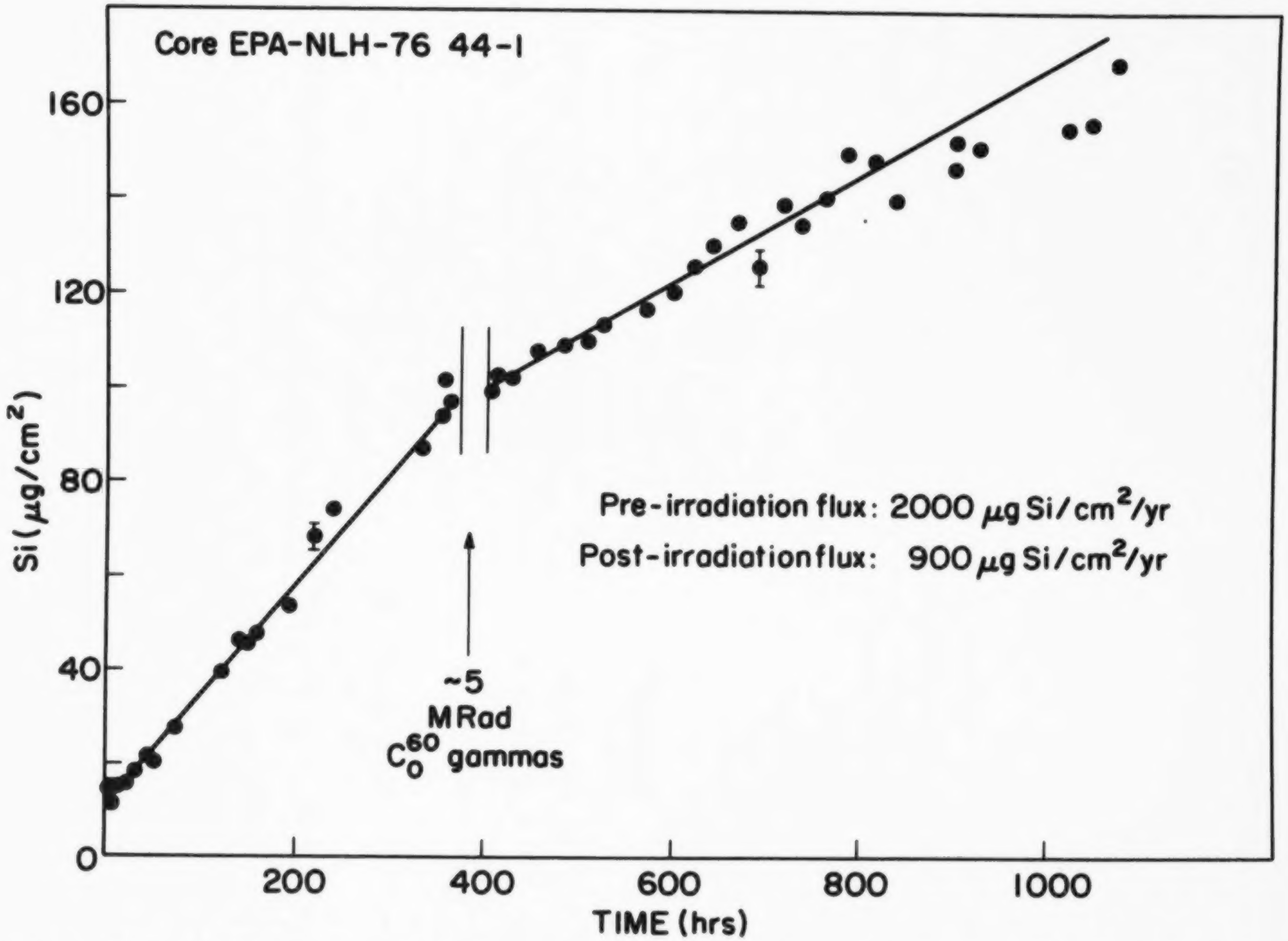

Figure 13. Flux of dissolved silicon from a sediment core collected from northern Lake Huron before and after exposure to a sterilizing dose of gamma radiation. Removal of the biological influence results in a significant reduction in the rate of dissolved silicon release.

TABLE 3. EFFECTS OF SELECTED TREATMENTS OF SILICA RELEASE FROM SEDIMENTS

Core	Treatment	Release Rate[1]	
		Before Treatment	After Treatment
NLH 2-11	Rotenone	.126 ± .021	.091 ± .020
NLH 2-14	Chlordane	.116 ± .016	.036 ± .010
NLH 4-4	Control	.136 ± .012	.086 ± .009
NLH 44-1	Gamma Radiation	.234 ± .010	.116 ± .013
LM 5-1	Tubificids	.241 ± .022	.177 ± .012
LM 5-3	Pontoporeia	.217 ± .015	.202 ± .016
LM 5-2	Control	.166 ± .017	.120 ± .014
NLH 44-3	Sediment Stirred at Coring	.0132 ± .016	---

[1] μg/cm /hr.

significant reduction in flux occurred following addition of rotenone (about 1 ml of saturated solution in ethyl alcohol). Note that reduction of the flux in the control cell reflects the progressive approach toward an equilibrium concentration of silicon in overlying water. In another set of experiments, tubificid worms and Pontoporeia were added to cores so as to increase the natural population densities by about a factor of two. As can be seen in Table 3, these additions did not result in a significant increase in the silicon flux. In retrospect, it appears likely that the addition of Chironomid larvae would have produced the increase in the flux.

Our results suggests an important role for benthos in the cycling of silica (and possibly other nutrients) in the Great Lakes. As silica is a major and probably limiting nutrient for the diatom productivity, it is important to understand the role of benthos and Chironomid larvae in particular in nutrient regeneration and the possible effect of aquatic pollutants on their interaction with sediments.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the help of Cheryl Hoyt, Karen Husby, Kjell Johansen, John Krezoski, and Maranda Willoughby in various aspects of field and laboratory work. Great Lakes Research Division Contribution Number 254.

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SECTION 16

RECENT ADVANCES IN THE STUDY OF NITRITE TOXICITY TO FISHES

Rosemarie C. Russo[1]

Nitrite has not until recently received much attention as a toxicant to aquatic organisms. However, it has been established that nitrite is very toxic to fishes and aquatic invertebrates. Furthermore, nitrite has been implicated in the formation of *N*-nitroso compounds (Archer *et al*. 1971; Wolff and Wasserman 1972; Mirvish 1975), and nitrosamines have been shown to be carcinogenic to zebra fish (*Brachydanio rerio*), rainbow trout (*Salmo gairdneri*), and guppy (*Lebistes reticulatus*) (Stanton 1965; Ashley and Halver 1968; Sato *et al*. 1973). Recently nitrite has been reported to induce cancer in rats directly, rather than through formation of nitrosamines (Newberne 1979).

In the past few years much research has been done to investigate the toxicity of nitrite to aquatic organisms. This includes the study of nitrite toxicity to additional fish species, the effects of water chemistry conditions on nitrite toxicity, and some work on the mode of toxic action of nitrite.

Nitrite is produced as an intermediate product in the nitrification process. In this process, the biological oxidation of ammonia to nitrate, *Nitrosomonas* bacteria convert ammonia to nitrite, and *Nitrobacter* converts nitrite to nitrate. The effectiveness of the conversion process is affected by several factors, including pH, temperature, dissolved oxygen concentration, numbers of nitrifying bacteria, and presence of inhibiting compounds. Under normal circumstances the first conversion, ammonia to nitrite, is the rate-limiting step in the process; the second conversion, nitrite to nitrate, is relatively rapid. For this reason, nitrite is generally present in only trace amounts in most natural freshwater systems. In sewerage treatment plants utilizing the nitrification process, the process may be impeded, causing discharge of nitrite at elevated concentrations into the receiving water. Also, water reuse systems using the nitrification process may malfunction, resulting in increased nitrite levels in the treated water.

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It has been demonstrated (Anthonisen et al. 1976) that the nitrification process can be inhibited in the presence of nitrous acid (HNO_2) and un-ionized ammonia (NH_3). The total ammonia in a wastewater treatment system is present as ammonium ion (NH_4^+) and un-ionized ammonia (NH_3). If the pH of the solution increases, either naturally or by addition of a base, the concentration of un-ionized ammonia will increase. Un-ionized ammonia inhibits nitrobacters at concentrations (0.1-1.0 mg/l NH_3) appreciably lower than those (10-150 mg/l) at which it inhibits nitrosomonads. This impedes the conversion of nitrite to nitrate, causing nitrite to accumulate. When the pH decreases, as ammonium and nitrite are oxidized, an increase in nitrous acid (HNO_2) concentration occurs. Nitrous acid inhibits both nitrobacters and nitrosomonads at concentrations between 0.22 and 2.8 mg/liter. This inhibition of the process can also result in an increase in nitrite.

Several organic compounds likely to be found in significant concentrations in industrial wastes have been shown to inhibit the nitrification process (Hockenbury and Brady 1977). Dodecylamine, aniline, and *n*-methylaniline at concentrations less than 1 mg/liter caused 50 percent inhibition of ammonia oxidation by *Nitrosomonas*; *p*-nitrobenzaldehyde, *p*-nitroaniline, and *n*-methylaniline at concentrations of 100 mg/liter inhibited nitrite oxidation by *Nitrobacter*.

The loss of nitrification flora, especially resulting from the use of antibiotics, has also been indicted (Patrick et al. 1979) as a potential cause of large amounts of nitrite accumulating in natural waters.

In view of these considerations, nitrite may be present under some circumstances in natural waters at concentrations high enough to be deleterious to freshwater aquatic life. Some field data have been reported documenting this. Klingler (1957) has reported nitrite concentrations of 30 mg/liter nitrite-nitrogen (NO_2-N) and higher in waters receiving effluents from metal, dye, and celluloid industries. McCoy (1972) has reported concentrations up to 73 mg/liter NO_2-N in Wisconsin lakes and streams. We have observed levels of 0.1 mg/liter NO_2-N in a reasonably clean cold water trout stream in Montana (Russo and Thurston 1974).

The literature through 1977 on nitrite toxicity to fishes has been summarized elsewhere (Russo and Thurston 1977, 1978; U.S. EPA 1977). Most of the data available do not include 96-hour LC50 values, but some comparisons can be made. From this and more recent literature there appear to be some differences, at least on a short term (less than four days) basis, in the relative susceptibilities to nitrite of different fish species. Concentrations as low as 0.2 mg/liter NO_2-N are acutely lethal to several species, with trout and salmon being the most susceptible. Concentrations in the range of 2 to 15 mg/liter NO_2-N have been reported to be lethal to some warmwater species, such as fathead minnows (*Pimephales promelas*) and channel catfish (*Ictalurus punctatus*). Some fish species, such as creek chub (*Semotilus a. atromaculatus*) and carp (*Cyprinus carpio*), succumb only at higher concentrations, up to 100 mg/liter NO_2-N. Of the fish species studied, those most tolerant to nitrite were: common white sucker (*Catostomus commersoni*), quillback (*Carpiodes cyprinus*), and mottled sculpin (*Cottus bairdi*). These species incurred no mortalities during short exposures to NO_2-N concentrations of 67 to 100 mg/liter. Manifestations of the acutely toxic effects of nitrite can thus vary widely, depending on fish species.

Little information has been reported on the effects of nitrite exposure for periods of time longer than 1-4 days. We have conducted 36-day exposures on cutthroat trout (S. clarki) fry (Thurston et al. 1978) and found LC50 values at 36 days to be only slightly lower than 96-hour values. Wedemeyer and Yasutake (1978) exposed steelhead trout (S. gairdneri) to low NO_2-N concentrations (0.015-0.060 mg/liter) over a 6-month period and found no serious deleterious effects. Growth and ability of the fish to adapt to seawater were not impaired. Varying degrees of gill hyperplasia and lamellar separation were observed early in the test but the fish seemed to recover and after 28 weeks these abnormalities were no longer observed.

Fish size has also been thought to be a factor influencing fishes' susceptibility to nitrite. Rainbow trout sac fry, and 2-g fry, were found to be less susceptible than were larger (12-, 14-, and 235-g) rainbow trout (Russo et al. 1974); 4.5-g fingerling rainbow trout were reported to be more tolerant than were 100-g yearlings (Smith and Williams 1974). Coho salmon (Oncorhynchus kisutch) fry (0.65 g) were less susceptible than were yearlings (22 g) (Perrone and Meade 1977). We have now conducted 20 96-hour nitrite bioassays on rainbow trout over the size range 2 to 387 g. These experiments were all conducted under similar water chemistry conditions (Table 1). The results are given in Table 2; over this larger range of fish size than that reported previously, there does not appear to be any relationship between fish size and susceptibility to nitrite. This is illustrated in the graphs of LC50 vs. fish weight and length, shown in Figures 1 and 2.

We have also studied the effect of chloride ion (Cl^-) on nitrite toxicity to rainbow trout (Russo and Thurston 1977). We conducted a series of nitrite toxicity tests in which we added Cl^- (as NaCl) in concentrations ranging from 1 to 41 mg/liter. A significant reduction in nitrite toxicity resulted from increased levels of Cl^- (Figure 3), and this effect was linearly correlated (Figure 4). The 96-hour LC50 was raised from 0.46 mg/liter NO_2-N in the presence of 1 mg/liter Cl^- to 12.4 mg/liter NO_2-N at 41 mg/liter Cl^-. Similar conclusions have been reported for coho salmon (Perrone and Meade 1977) and for steelhead trout (Wedemeyer and Yasutake 1978). We have conducted some nitrite bioassays with addition of bromide (Br^-), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), and nitrate (NO_3^-); the results of these tests indicate that these other anions also exhibit, in different degrees, an inhibitory effect on nitrite toxicity. It is apparent that the toxicity of nitrite is highly dependent on the chemical composition of the water.

Crawford and Allen (1977) studied the effect of calcium (Ca^{2+}) and of seawater on nitrite toxicity to chinook salmon (O. tshawytscha). The acute toxicity of nitrite in seawater was markedly less than that in freshwater, logically so because of the chloride effect discussed above. Crawford and Allen also found that increasing the calcium concentration both in freshwater and in seawater decreased the toxicity of nitrite.

TABLE 1. CHEMICAL CHARACTERISTICS OF THE DILUTION WATER USED IN BIOASSAYS. (ALL VALUES ARE MG/LITER UNLESS OTHERWISE NOTED)

Alkalinity, as $CaCO_3$	171	Al	<1
Hardness, as $CaCO_3$	200	As	0.0012
pH	7.70	Ca	52.1
Temperature, C	9.8	Cd	<0.005
S.E.C., µmho/cm 25 C	339	Cr	<0.005
TOC	3.3	Cu	0.007
Turbidity, NTU	1.6	Fe	0.004
NH_3-N	0.00	Hg	0.00030
NO_2-N	0.00	K	0.82
NO_3-N	0.14	Mg	16.7
Cl^-	0.16	Mn	0.002
F^-	0.35	Na	2.5
PO_4^{3-}	0.05	Ni	<0.005
SO_4^{2-}	17.2	Pb	<0.015
		Se	0.00085
		Zn	0.01

TABLE 2. ACUTE TOXICITY OF NITRITE TO RAINBOW TROUT (*SALMO GAIRDNERI*) UNDER UNIFORM WATER CHEMISTRY CONDITIONS

Test Number	Average Fish Size		96-hour LC50 (95% C.I.)
	Wt.(g)	Length(cm)	(mg/l NO_2-N)
117	2.3	--	0.38 (0.34-0.43)
579	3.1	6.3	0.25 (0.21-0.30)
585	7.0	9.1	0.40 N.C.[1]
587	8.0	8.6	0.36 (0.33-0.39)
590	8.2	8.7	0.30 (0.26-0.34)
182	8.8	8.8	0.14 (0.12-0.16)
597	10.0	9.3	0.21 (0.19-0.24)
600	10.4	9.2	0.17 (0.15-0.20)
120	11.9	--	0.21 (0.18-0.24)
121	12.1	--	0.21 (0.19-0.23)
605	12.8	10.3	0.21 (0.19-0.24)
610	13.2	10.3	0.22 (0.18-0.27)
102	14.0	--	0.26 (0.21-0.32)
323	20.6	11.8	0.27 N.C.
326	24.3	12.3	0.28 (0.24-0.32)
243	53.1	15.7	0.27 (0.22-0.32)
244	60.5	16.6	0.27 (0.23-0.32)
423	188	23.6	0.19 (0.15-0.24)
138	235	--	0.20 (0.16-0.24)
505	387	29.7	0.24 (0.17-0.33)

[1] N.C. = Confidence interval not calculable.

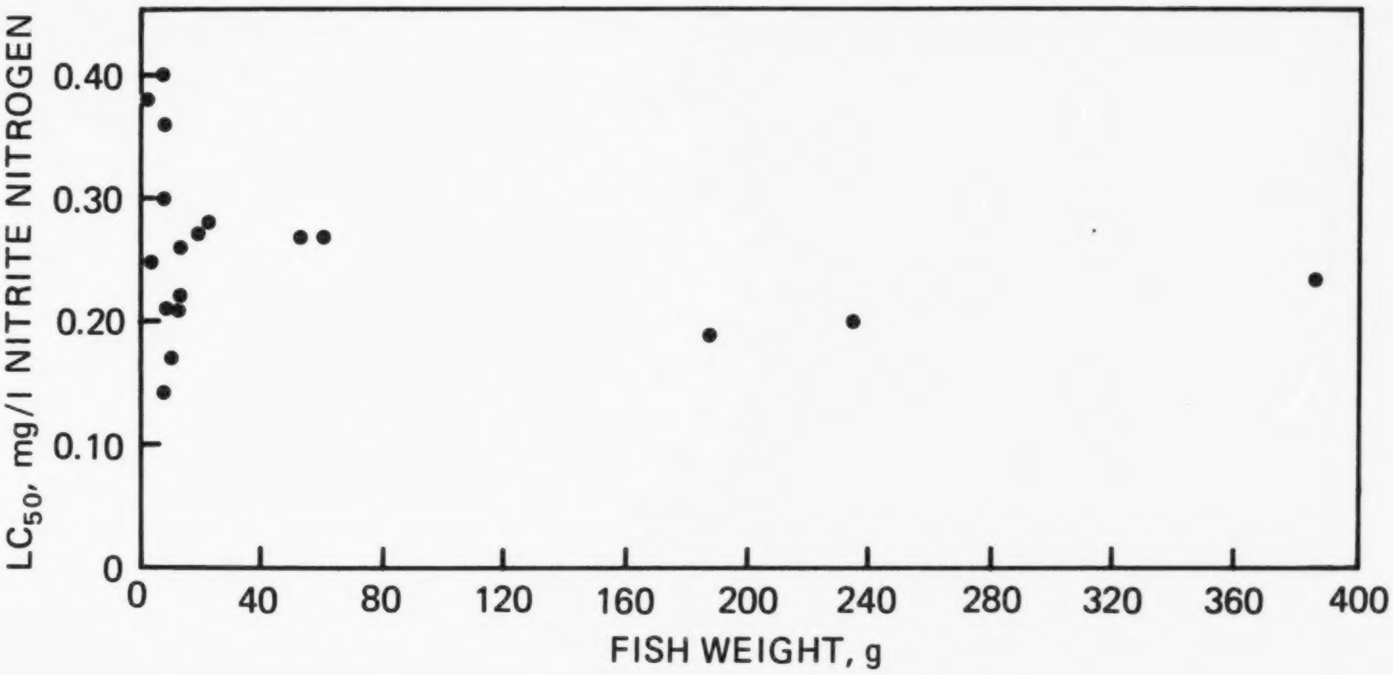

Figure 1. LC50 vs. average fish weight for nitrite bioassays on rainbow trout (Salmo gairdneri).

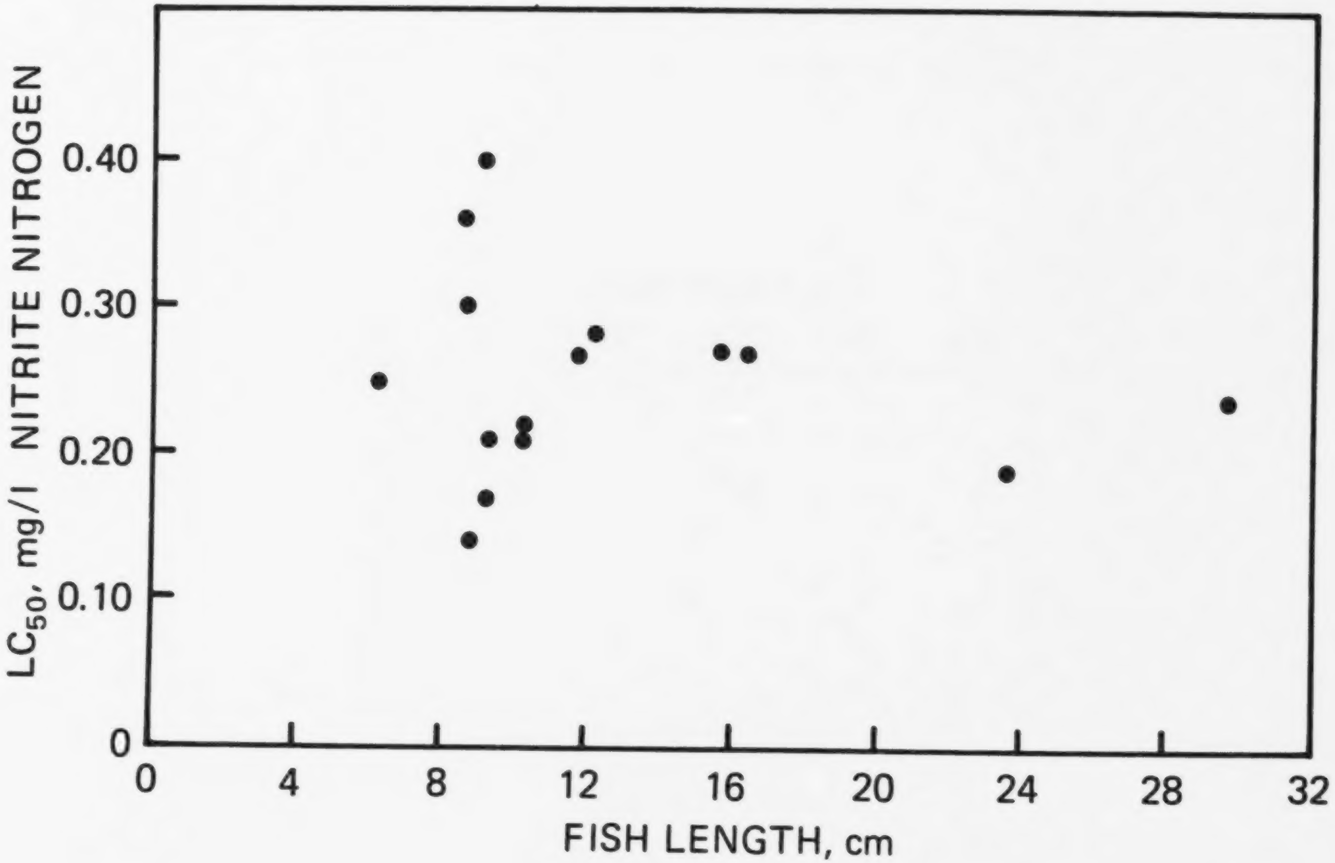

Figure 2. LC50 vs. average fish length for nitrite bioassays on rainbow trout (*Salmo gairdneri*).

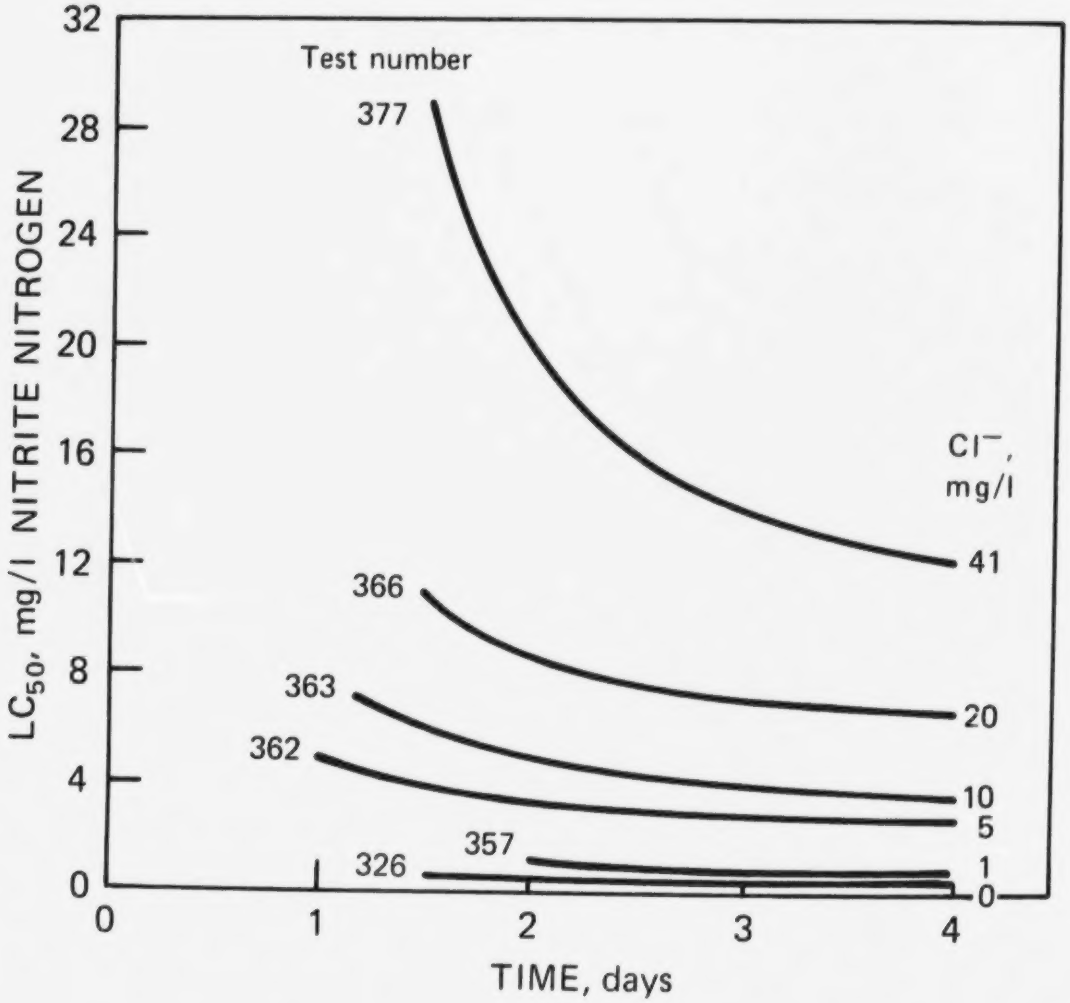

Figure 3. Toxicity curves showing effect of chloride on nitrite toxicity to rainbow trout (*Salmo gairdneri*).

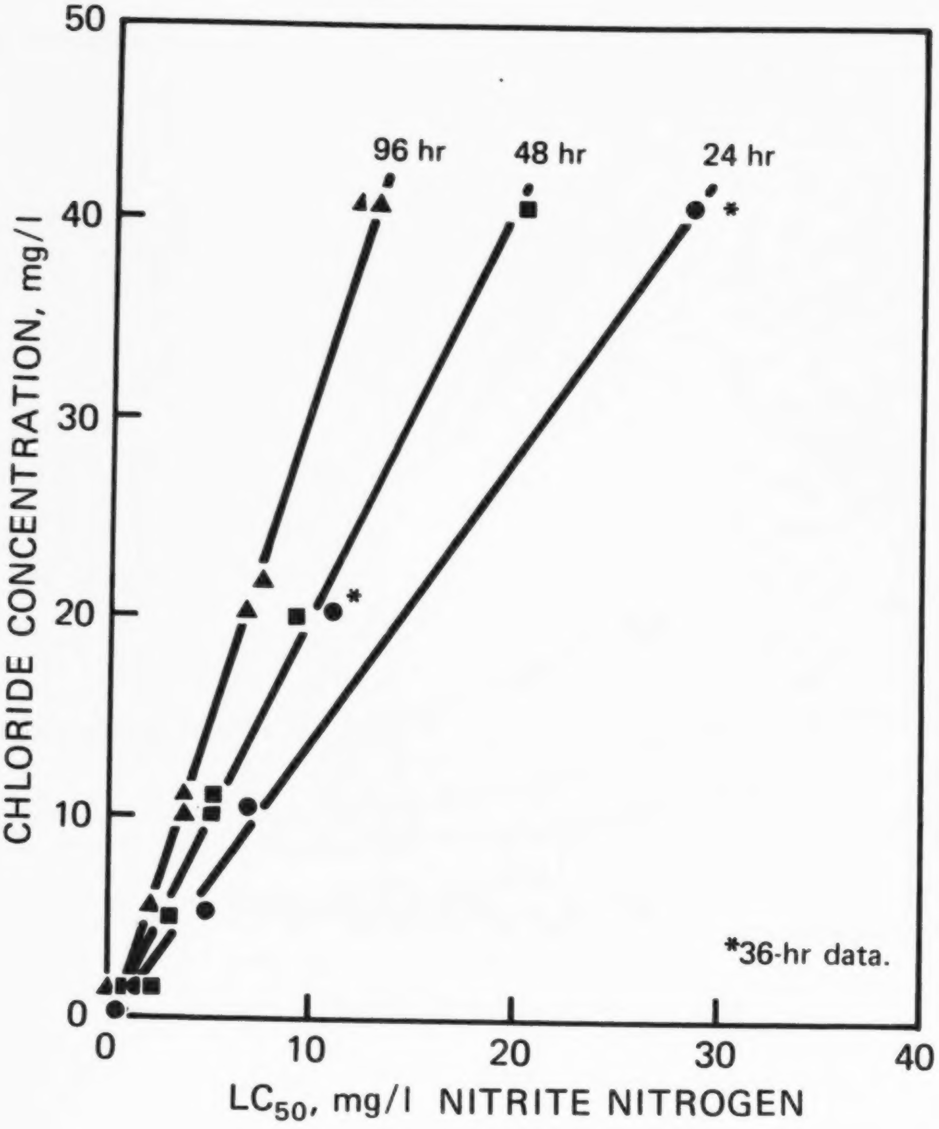

Figure 4. Effect of chloride on nitrite toxicity to rainbow trout (*Salmo gairdneri*).

An additional factor that should be considered in regard to nitrite toxicity is the pH of the solution. Nitrite ion establishes the following aqueous equilibrium.

$$NO_2^- + H^+ \rightleftarrows HNO_2$$

The concentration of nitrous acid (HNO_2) is 4-5 orders of magnitude less than the concentration of nitrite ion (NO_2^-) within the pH range 7.5 to 8.5; in going from pH 7.5 to 8.5, the NO_2^- concentration stays essentially constant, whereas the HNO_2 concentration decreases tenfold. Because this equilibrium is pH-dependent, we studied the toxicity of nitrite to rainbow trout over the pH range 6.4 to 9.0, to examine the effect of pH on nitrite toxicity and to see whether toxicity could be attributed to one or the other of the chemical species.

The results for a series of these experiments are shown in Figures 5 and 6. The first figure is a plot of 96-hour LC50 vs. pH for total NO_2-N. It shows that the toxicity of nitrite decreases with increasing pH. If the toxicity of nitrite were solely due to the NO_2^- ion, this plot would be a horizontal line. The second figure shows a plot of LC50 vs. pH for nitrous acid (as N). If all the toxicity were attributable to this nitrite species, this plot would be horizontal. Neither plot is horizontal, suggesting that neither chemical species alone is responsible for the entire toxicity. Over the pH range studied, both species are significantly, although not necessarily equally, toxic. It is not possible to separate the toxicity into its components without additional data, but in order to obtain these data by the design we chose, experiments would have to be carried out beyond the pH range acceptable for fishes.

The question of mode of toxic action of nitrite on fishes has also been studied. Oxygen is transported in fish blood by the respiratory blood pigment hemoglobin. The iron in hemoglobin is present in the ferrous, Fe(II), state. Hemoglobin combines loosely with oxygen to form the easily dissociated compound oxyhemoglobin, in which iron is still in the Fe(II) state. The transport of oxygen by blood is dependent on the ease with which hemoglobin unites with oxygen and with which oxyhemoglobin gives up oxygen. If the iron in hemoglobin is oxidized to the ferric, Fe(III), state, methemoglobin is formed. Methemoglobin is not capable of combining reversibly with oxygen, and thus sufficiently high concentrations can cause hypoxia and death. Nitrite in the blood oxidizes hemoglobin to methemoglobin, thereby increasing the amount of methemoglobin present and impairing the ability of the blood to transport oxygen.

It has been established that increased nitrite concentrations produce increased methemoglobin levels in fish blood (Smith and Williams 1974; Smith and Russo 1975; Brown and McLeay 1975; Crawford and Allen 1977; Perrone and Meade 1977; Bortz 1977). The presence of high levels of methemoglobin in fish blood is visually apparent in that the blood becomes brown-colored. Different levels of methemoglobin have been reported as the concentrations causing mortality in fishes. Species differences and differences in overall physical condition may influence fishes' tolerance to different methemoglobin levels.

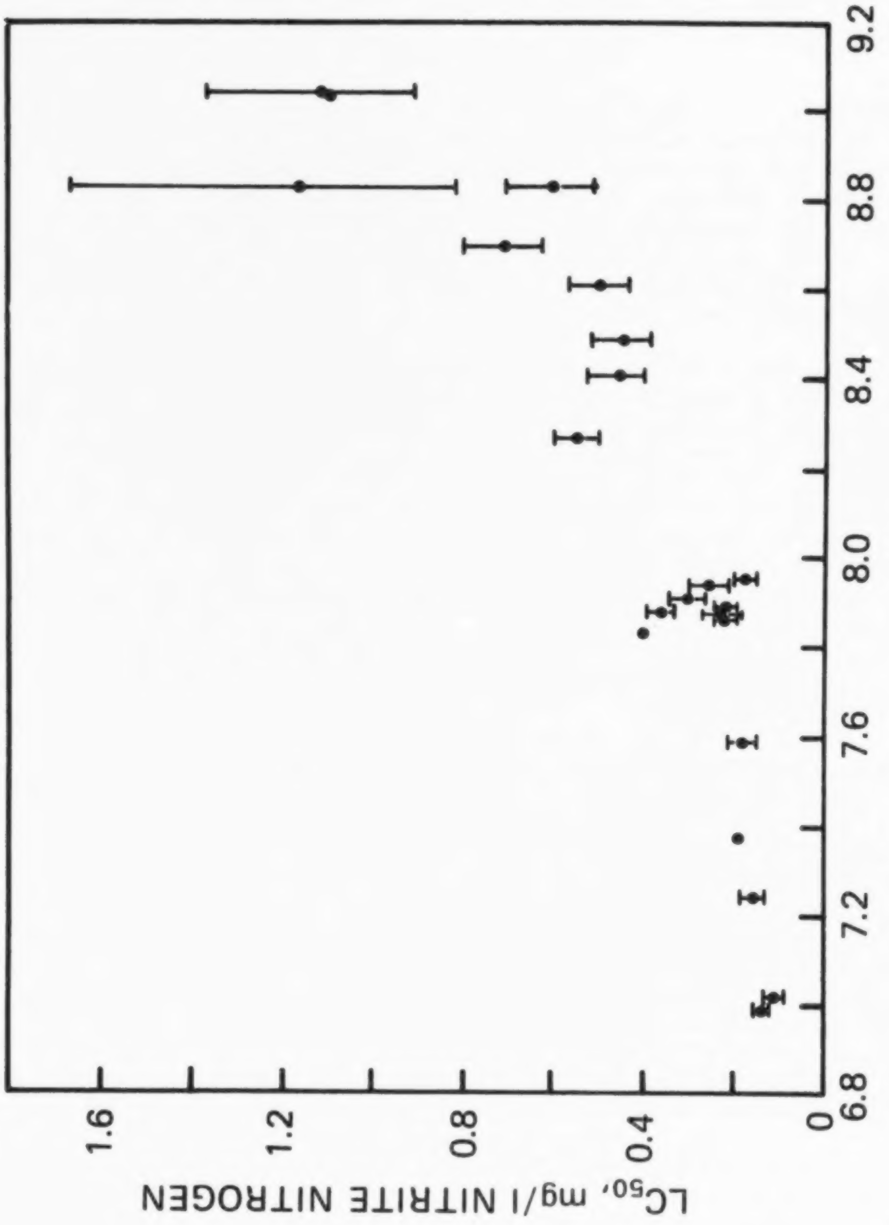

Figure 5. LC50 (as NO_2-N) vs. pH.

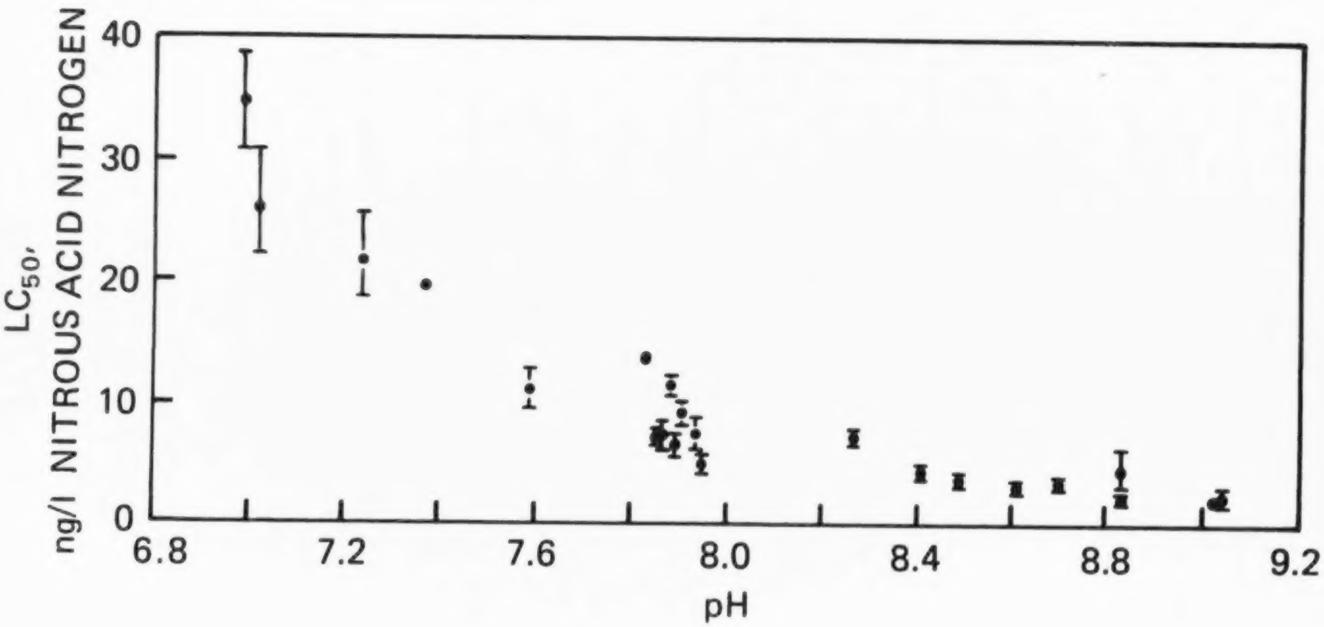

Figure 6. LC50 (as HNO_2-N) vs. pH.

Some work has been done on treatment of methemoglobinemia. Ascorbic acid administered intravenously reduced methemoglobin in rainbow trout blood (Cameron 1971). Methylene blue administered either by injections (Bortz 1977) or by addition to test water (Wedemeyer and Yasutake 1978) also reduced methemoglobin levels. Removal of fish to nitrite-free water results in a reduction of methemoglobin levels, although to a smaller extent than found for methylene blue treatment (Wedemeyer and Yasutake 1978). Methylene blue reduces methemoglobin levels rapidly, within a few hours. The treatment appears to be temporary, in that methemoglobin levels gradually rise again (Bortz 1977).

Methemoglobinemia, then, is one mechanism by which nitrite is toxic to fishes. It is probably not the only mode of toxic action. Observations by Smith and Williams (1974) that mortality occurred for some rainbow trout with blood methemoglobin levels lower than other rainbow trout which survived led them to suggest that those fish died from a toxic reaction to nitrite itself rather than from methemoglobinemia. Crawford and Allen (1977) observed that in seawater with added nitrite, chinook salmon had high (74%) methemoglobin levels but very low (10%) mortality; in freshwater with added nitrite, lower (44%) methemoglobin levels were found in the salmon, but 70% mortality occurred. They further observed that fish dying in freshwater often had red gill lamellae, not the brown color typically caused by methemoglobinemia. This indicates that the toxicity of nitrite in freshwater may be attributable to something else besides or in addition to methemoglobinemia. More research is needed to determine what this mechanism is.

The effect of chloride and calcium also needs more study to elucidate the mechanism by which these ions reduce nitrite toxicity. It has been suggested (Perrone and Meade 1977) that chloride may compete with nitrite for uptake through gills, or for entry into the red blood cell, thus suppressing methemoglobin formation. Calcium does not appear to be affecting methemoglobin formation, because raising the calcium level of freshwater did not reduce methemoglobin levels in chinook salmon (Crawford and Allen 1977). These are important areas for further research.

In conclusion, it is apparent that the toxicity of nitrite to fishes is highly dependent on the chemical composition of the test water, and that more research is needed to define the mechanism(s) of nitrite toxicity and to learn more about ways to protect fish from nitrite poisoning.

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